

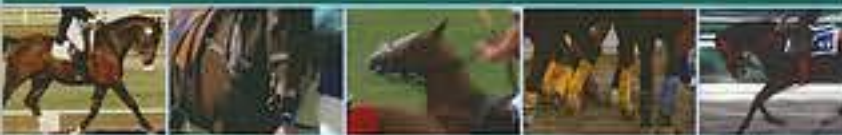
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EQUINE EXERCISE PHYSIOLOGY

The Science of Exercise
in the Athletic Horse



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Raymond J. Geor
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Preface

We believe that a good understanding of the physiology of exercise is important for anyone involved in the care, handling, training, feeding, breeding, or use of horses. The care of horses, and athletic horses in particular, requires an understanding of the physiological demands of exercise, which then provides a better grasp of the requirements of competition and training. The science of equine exercise physiology has progressed to the stage that it now provides a sound, scientific basis for much of the nutrition, training, and care of equine athletes. The current level of knowledge, while still incomplete and imperfect, of the physiological processes underlying the acute responses to exercise and the mechanisms and effects of exercise conditioning, provides a sound, fundamental understanding of the workings of the equine athlete. Contemporary equine exercise physiology comprises not only the physiological responses to exercise and training, but also nutrition, biomechanics, behavior, and pharmacology. This fundamental knowledge informs our decisions regarding appropriate training, nutrition, care, and treatment of the equine athlete.

The first edition of *Equine Exercise Physiology* is a response to requests by many readers of *Equine Sports Medicine and Surgery: basic and clinical sciences of the equine athlete* that we provide a text focused on equine exercise physiology. This text is intended for anyone involved with athletic horses, and in particular those individuals who are not responsible for providing the primary veterinary care of such horses. While the text presupposes a good knowledge of physiology, we think that it is written at a level that will allow most informed individuals to gain much valuable information.

We thank the colleagues and students with whom we have had the pleasure of working and who provided much of the knowledge contained within this book. Our profound gratitude is extended to the authors of sections of this book with an appreciation of the effort that was required to compile anew comprehensive material on their designated topic. We hope that they, and the readers, are pleased with the final product.

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The horse as an athlete: a physiological overview

Kenneth W. Hinchcliff & Raymond J. Geor

The horse as an athlete

Comparative physiology

The horse is an extraordinary athlete, a characteristic that is the result of the evolution of horses as grazing animals on the ancient prairies of North America. Survival in these open lands was enhanced by speed, to escape predators, and endurance, required to travel long distances in search of feed and water. These attributes are shared by pronghorn antelopes, another species that evolved on the prairies. The equid characteristics of speed and endurance were subsequently modified or enhanced by selective breeding by humans.

Horses were domesticated on more than one occasion, based on analysis of mitochondrial DNA from a wide variety of current domestic breeds and Przewalski's horses.^{1,2} Domesticated horses were then selected and bred for certain traits, depending on the intended use, and this has resulted in some breeds, notably Thoroughbreds and American Arabs but likely also other breeds, having a narrow genetic base.^{3,4} Ten founder mares account for 72% of maternal lineages, and one founder stallion accounts for 95% of paternal lineages among Thoroughbred horses. The result of selective and close breeding (breeding related horses to each other) is the formation of a variety of breeds of horse with widely varying body types.

Horses have been bred or adapted to a large variety of uses. Large, heavy breeds of horse were bred for draft work, such as pulling plows, sleds, or carts, or military work, such as the chargers that carried heavily armored knights into the battles of the Middle Ages. Lighter horses were bred for speed and endurance and were used for transportation, herding, and sport. Thoroughbred race horses run at high speed (18 m/s, 64 km/h) over distances of 800–5000

meters. Standardbred horses trot or pace at high speed for distances up to 3600 m. Quarter horses sprint for 400 m or less at speeds as high as 88 km/h (see Chapter 4.1), sometimes around figure of eight courses delineated by barrels (barrel racing), and Arabians trot or canter for up to 160 km in a single day during endurance events (and over longer distances during multi-day races). In contrast, draft horses pull huge weights (1000 kg or more) short distances in pulling competitions. Warmbloods perform elegant, but demanding, dressage routines, and ponies pull lightly laden jinkers or buggies.

Regardless of their size, provenance or intended use, all horses have in common an ability to perform physical activities, including running or jumping, at a level that surpasses that of most other animals of similar body size. The concept of body size is important, as many physiologic variables, and especially the maximum values of these variables, do not scale directly with bodyweight but often more closely scale to an exponent of bodyweight.⁵ Commonly, exponents range between 0.68 and 0.75. This exponent is derived empirically from the measurement of variables such as maximum running speed or maximum rate of oxygen consumption ($\dot{V}O_{2\max}$). Typically, when expressed as a one-to-one function of bodyweight (i.e. per kg), values for many variables are much higher for smaller mammals. The necessity to scale variables allometrically has fascinating physiologic implications and interpretations.⁵ However, direct comparison among species is to some extent specious from the point of view of depicting differences in physical capacity, given that the absolute values of these variables vary to such a large extent. Nevertheless, such comparisons are frequently made, if only to reinforce the magnitude of the maximal absolute values of these variables in the exercising horse (Table 1.1.1).

Table 1.1.1 Selected physiological variables of athletic and non-athletic species

Species	Bodyweight (kg)	Speed ^a (km/h)	Duration of exercise	$\dot{V}O_{2\max}$ (mL O ₂ /kg/min)	Heart rate ^a (bpm)	Energy expenditure per day (kcal)
Thoroughbred race horse	450	64 (max)	2 min	180–200	240 (max)	30 000
Endurance race horse	400	15–35	6–8 h	180		38 000 ^b
Steer	470			80		
Goat	32					
Greyhound	34	64 (max)	60 s	Not reported	300	2160
Sled dog	25	20	10 days	170	300	11 000
Human (Olympic class sprinter)	70	36 (max)	9.4 s	85	220	
Human (Olympic class endurance)	70	19	2 h	70	180	2300 ^c , 7000 ^d
Pronghorn antelope	32	65	10 min	300		

^aDuring customary athletic activity.^bDay of racing.^cDuring marathon race.^dTour de France cyclists.

max = maximum value.

Physiologic attributes of horses

The athletic capacity of horses is attributable to a number of physiologic adaptations. In some cases these adaptations are not affected by training — for example, lung size, whereas others change in response to training — for example, blood volume (see Chapters 3.2 and 6.1). The superior athletic ability of horses is attributable to their high maximal aerobic capacity, large intramuscular stores of energy substrates and in particular glycogen, high mitochondrial volume in muscle, the ability to increase oxygen-carrying capacity of blood at the onset of exercise through splenic contraction, efficiency of gait, and efficient thermoregulation.

High maximal aerobic capacity

The maximal aerobic capacity ($\dot{V}O_{2\max}$) of horses is approximately 2.6 times that of similarly sized cattle, and on a bodyweight basis is approximately 2–2.5 times that of highly trained men.⁶ Horses have structural adaptations that enhance oxygenation of blood in the lungs, oxygen transport capacity of blood, and the ability to deliver oxygen to tissues. The oxygen transport chain, from air to muscle, of horses is suited to transportation of the large volumes of oxygen

required to support the high metabolic rate of strenuously exercising horses. The large aerobic capacity in horses is associated with a number of factors including a larger maximum cardiac output and higher hemoglobin concentration.⁶

Cardiac output is the product of heart rate and stroke volume, and it is the cardiac output that determines the delivery of blood, and hence oxygen, to tissue. Cardiac output of Thoroughbred horses during intense exercise can be as high as 400 L/min. Maximum heart rate is not different between horses, cattle, and men. Horses, with a heart weight of 0.9–1.1% of bodyweight, have proportionately large hearts and therefore stroke volume, thereby explaining the large cardiac output in this species. The heart weight of Secretariat was estimated to be 10 kg, whereas that of stallions of lesser quality was up to 50% less (see Chapter 4.1). The heart size as a proportion of bodyweight varies among various breeds of horses, with draft breeds having proportionately much smaller hearts than those of racing breeds (see Chapter 4.1).

Oxygen transport depends on the ability to transfer oxygen from the air to pulmonary capillary blood. This involves movement of air from outside the horse to the alveoli, and subsequent diffusion of oxygen into

the pulmonary capillary. Thoroughbred horses have tidal volumes of approximately 12 liters and minute ventilation of 1600 L/min during maximal exercise on a treadmill (see Chapter 3.1). These extraordinarily high flow rates are generated by pressure differences between the pleural space and ambient air. Generation of alternating negative and positive pressure in the thorax during exercise is the result of a complicated interaction between locomotory and respiratory muscles (see Chapter 3.1). Diffusion of oxygen from the alveolar lumen to the capillary blood is achieved because of the thin membrane separating the alveolus from capillary blood, and the large surface area of lungs of horses. Horses have lungs that are twice as large as those of cattle, with gas exchange surface area 1.6 times that of cattle.⁷

Oxygen transport from the lungs to exercising muscle is achieved by the circulation. In addition to cardiac output, oxygen delivery is limited by the oxygen-carrying capacity of blood. Horses achieve rapid increases in the oxygen-carrying capacity of blood by increasing hemoglobin concentration in blood through splenic contraction. Splenic contraction in anticipation of exercise and during exercise increases the circulating red cell mass without concomitant increases in plasma volume.⁸ The resulting increase in hemoglobin concentration increases the oxygen-carrying capacity of arterial blood by up to 50% during intense exercise. The effect of the spleen on hemoglobin concentration is supplemented by a reduction in plasma volume during intense exercise.⁹ The beneficial effect of this autotransfusion of red cells at the start of exercise is apparent in horses from which the spleen has been removed.^{10–12} Splenectomized horses have lower hematocrits during exercise, altered systemic hemodynamics including lower right atrial and pulmonary artery pressures, and reduced capacity to perform strenuous exercise.

The final step in the oxygen transport process is the utilization of oxygen in muscle. High rates of aerobic metabolism in muscle of horses are favored by the high capillary density in muscle, thus favoring oxygen delivery, and high activity of enzymes involved in energy utilization in muscle (see Chapter 2.1). Mitochondria provide the energy for muscle contraction. The greater the quantity of mitochondria per unit of muscle weight, the greater is the oxidative capacity of muscle. Muscle of horses contains approximately twice the concentration of mitochondria as does muscle of cattle, a similarly sized animal but with a much lower aerobic capacity.¹³ This greater aerobic capacity in muscle, when supported by adequate

substrate availability and oxygen delivery, permits a higher whole animal maximal aerobic capacity.

Energy metabolism

The oxidation of CHO and fat, with perhaps a minor contribution by protein (amino acids), provides the chemical energy for muscle contraction. Glucose is stored as glycogen in liver and skeletal muscle, with more than 90% of total body CHO stores in muscle. Fat is stored in adipose tissue and muscle, with the majority (>85%) in adipose tissue. The fat store is considerably larger when compared to the CHO reserve (50–60-fold more energy as fat). As such, CHO stores are relatively limited when compared to fat. Within muscle, fat is stored at both intracellular (myocellular) and extracellular sites. There is differential storage of glycogen and intramyocellular triglycerides (IMTG) in the different skeletal muscle fiber types. Highly oxidative type I fibers have greater lipid storage than fast-twitch, high oxidative fibers (type IIA), while there is negligible triglyceride in fast-twitch, low oxidative fibers (type IIX). The reverse is true for glycogen content. The glycogen concentration in horse muscle (homogenate of middle gluteal m.) is approximately 130–150 mmol/kg wet weight (ww) (550–650 mmol/kg dry weight (dw)),^{14,15} which is considerably higher when compared to sled dogs (70–80 mmol/kg ww or 330–350 mmol/kg dw) or humans (80–140 mmol/kg ww).^{16–19} On the other hand, the IMTG content of hindlimb locomotory muscles may be higher in dogs than in horses. Electron microscopic studies in dogs and horses have indicated that dogs have a threefold higher quantity of lipid droplets in oxidative fibers, although reliable estimates of intramuscular lipid concentration in horses are not available.¹¹ In summary, horses have large stores of intramuscular substrate that is readily available for use during exercise, with glycogen being the most important substrate.

The relative contributions of CHO and fat to energy use depend on several factors, including exercise intensity, training state, muscle composition, diet and feeding state, and the duration of exercise. As the intensity of exercise increases, there is an increase in the energy contribution from CHO oxidation, primarily muscle glycogen, and a decrease in the contribution from fat oxidation. This shift in substrate metabolism with increasing intensity of exercise has been termed the *crossover concept*. The pattern of substrate utilization also varies with exercise duration; during prolonged submaximal exercise there is a progressive increase in fat oxidation with a concomitant decrease in the energy contribution from CHO

oxidation that parallels a decrease in CHO (muscle glycogen) stores. These general patterns of substrate utilization are important when considering the endurance capacity of horses and dogs. Specifically, the duration of high-intensity exercise that demands a high rate of CHO oxidation is limited by CHO availability in skeletal muscle. Conversely, an exercise intensity that allows for a substantial energy contribution from fat oxidation may be sustained for a longer duration. In dogs and humans, the rate of fat oxidation peaks at a work intensity corresponding to approximately 40–50% of $\text{VO}_{2\text{ max}}$.²⁰ In horses, the work intensity corresponding to the maximum rate of fat oxidation is not known. However, in one treadmill study²¹ there was a decrease in the estimated rate of fat oxidation when exercise intensity was increased from ~30% to ~60% $\text{VO}_{2\text{ max}}$, suggesting that maximal values are attained at a similar or even lower relative work intensity when compared to dogs and humans. Indeed, there is evidence that dogs utilize a higher proportion of fat vs. CHO during submaximal exercise when compared to horses. During treadmill running at ~60% $\text{VO}_{2\text{ max}}$ the relative contributions to energy expenditure from CHO and fat were, respectively, 60% and 40% in dogs,²⁰ and 75% and 25% in horses.²¹ Thus, during moderate-intensity exercise, horses appear to be more dependent on CHO oxidation than dogs. This difference in the pattern of substrate utilization may be related to variation in muscle composition and substrate storage (i.e. glycogen vs. triglyceride).

The relevance of these data to field (i.e. racing) conditions requires knowledge of the relative work intensities of horses during various forms of exercise, including racing. Unfortunately, these data are not available. However, based on published values for $\text{VO}_{2\text{ max}}$ and the relationship between running speed and oxygen consumption (or heart rate vs. VO_2), it is possible to estimate energy expenditure and the relative contributions from oxidation of CHO and fat in horses during sustained exertion. The average running speed of elite endurance horses during races of 80–160 km is approximately 19–22 km/h (~5–6 m/s), which corresponds to a relative work intensity of approximately 50–55% of $\text{VO}_{2\text{ max}}$. Based on laboratory data, this workload would result in an energy expenditure of approximately 0.25–0.30 kcal/kg/min (~6500 kcal/h for a 450 kg horse) with relative contributions to energy of about 60% from CHO oxidation and 40% from fat oxidation. Assuming a linear relationship between work intensity or speed and energy expenditure, energy expenditure of horses running at

maximal speed is likely to be in the range of 0.50–0.60 kcal/kg/min, with almost all of this energy provided by oxidation of creatine phosphate, glucose, and glycogen. It is important to realize that during high-intensity exercise, which is of necessity of short duration, almost all energy is derived from creatine phosphate and, to a much larger extent, glycogen. At the speed at which endurance horses compete glycogen also provides the majority of energy, but fat provides an important source of energy. These estimates are at variance with the widely held belief that fat oxidation is the primary source of energy in horses during endurance exercise, and suggest that CHO availability in skeletal muscle may be an important determinant of performance in this species during sustained exertion, in addition to the demonstrated effect of glycogen depletion during intense exercise.²²

High intramuscular concentrations of substrate are important for fueling muscle contractions during exercise. The flux of glucose from blood into muscle and subsequently to the mitochondria provides only a small amount (<10%) of the energy used during intense exercise,²³ probably because of limits to the rate of transportation of these compounds during exercise.¹⁵ The presence of large amounts of readily available substrate in close proximity to mitochondria is therefore essential for horses to undertake strenuous exercise.

Gait

Energetically efficient gait is challenging for large animals because of the slow rate of contraction and low power output of their muscles.²⁴ However, the gait of horses is energetically efficient,²⁵ with the muscular work of galloping being halved by elastic storage of energy in muscle and tendon units.²⁶ For the forelimb, this use of stored energy and the subsequent catapult action mean that the biceps and brachiocephalicus muscles are less than one-hundredth the size that they would need to be were there no use of stored energy.²⁴ Horses are therefore biomechanically suited to energetically efficient locomotion. However, a complication of understanding the biomechanics of horses during intense exercise is the fact that many equine athletic events are not run in a straight line. Thoroughbred racing usually involves horses running on an elliptical track. While this has not been extensively studied in horses, greyhounds running around a bend have a 65% increase in peak force and a 70% increase in effective weight.²⁷ Whether this phenomenon occurs in horses, and the impact of such forces on energetic efficiency, are uncertain.

In summary, a large number of physiologic and anatomic features act in concert to endow the horse with extraordinary athletic capacity. Exceptional athletic performance is dependent upon optimal integrated functioning of these physiologic and anatomic features.

Genotype of successful horses

Athletic performance by horses is a heritable trait. This seems an obvious statement given that decades, and for some breeds centuries, of breeding have been aimed at selecting and breeding horses with superior performance based on the assumption that athletic capacity is heritable. Recent studies in Arabian, Thoroughbred, and Quarter horses have demonstrated the heritability of best racing time, race performance (as assessed by finishing position), and earnings.^{28–30} However, as with any trait that is likely to be polygenic and not controlled by a single gene, the heritability of performance characteristics is not particularly strong, reflecting the effect of environmental factors, including factors as early in life as the intrauterine environment, on future athletic capacity.³¹

There is considerable interest in identifying genes associated with performance in human athletes, and in identifying those variants (alleles) that are associated with either superior or inferior performance.³² The number of genes associated with performance in human athletes continues to increase each year, and is cataloged in an annual update.³³ The 2005 update listed 140 genes associated with performance or health-related fitness in humans.

Among the genes associated with superior athletic ability in humans are the I allele of the angiotensin-converting enzyme (*ACE*) gene and the *ATN3* gene which separately are associated with superior endurance performance, and the *ER22/23EK* polymorphism of the glucocorticoid receptor gene (*NR3C1*) which is associated with arm and leg muscle strength in men but not women.³³ At the time of writing, there are no reports of the association of athletic performance by horses with specific genes or alleles.

Integrative physiology of exercise

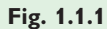
The detailed responses of each body system to acute exercise and to repeated exercise (conditioning or

training) are described in chapters throughout this book. These responses, although described in isolation for each body system, do not occur in isolation, but rather occur as a component of a complex and integrated response to exercise, the ultimate goal of which is to provide substrate for muscle contraction while maintaining homeostasis.

Exercise results in coordinated changes in almost all body systems. Fundamentally, exercise is associated with an increase in power output achieved by contraction of muscles. Contraction of muscles consumes adenosine triphosphate (ATP) and triggers an increase in metabolic rate to replace expended ATP. Increases in metabolic rate are dependent upon an adequate supply of substrate and, ultimately, oxygen. Energy production can be achieved for brief periods of time by anaerobic metabolism, but ultimately all energy production is linked to substrate oxidation and an adequate supply of oxygen.

Production of ATP during exercise is proximately dependent on supplies of substrate for oxidation and of oxygen. A schematic of factors influencing the supply of these fuels to muscle is depicted in Figure 1.1.1. The important concept is that there are a number of steps in the process or transport chain by which each of these products is delivered to the muscle cell. Because these processes are sequential and often non-duplicative, a limitation in one process or function will limit the rate of the whole system. In some cases these rate-limiting steps may be modified by training, in which instance the rate of oxygen or substrate delivery will be increased, or may not be altered by training. The consequences of these differences are discussed below under 'Factors limiting performance'.

At the onset of exercise there is a coordinated response by a large number of body systems to increase fuel availability, maintain acid–base balance within acceptable limits, and limit body temperature. These responses include a large increase in flux of substrate, the nature of which depends on the intensity and duration of exercise. Increasing exercise intensity results in a greater proportion of total energy production being derived from carbohydrates in muscle (glycogen) and blood (glucose, either absorbed from the gastrointestinal tract or produced by the liver) than from fat. The supply of these substrates is controlled by the hormonal responses to exercise, which include a reduction in blood insulin concentration and increases in blood catecholamine, cortisol and glucagon concentrations (see Chapter 5.2). The net result is increased delivery of glucose to muscle from the blood as a result of increased hepatic glycogenolysis



Aerobic metabolism and anaerobic glycolysis result in the production of waste products, principal among which are carbon dioxide and lactate. Carbon dioxide is produced by the aerobic metabolism of carbohydrate or fat. Produced in the mitochondria of metabolically active cells, it diffuses into the blood, wherein it is transported either as dissolved carbon dioxide or as bicarbonate (see Chapter 6.2). Transportation of the large amounts of carbon dioxide produced during exercise results in marked increases in venous partial pressure of carbon dioxide and venous blood bicarbonate concentrations.

Lactate, and associated H^+ , are produced during anaerobic metabolism. A 3-carbon monocarboxylate compound, lactate moves out of muscles and into other tissues by diffusion and by active transport by monocarboxylate transporters. Lactate is metabolized to carbon dioxide and water in well-oxygenated metabolically active tissues, or is recycled to glucose and glycogen in the liver, kidney and inactive muscle cells. The hydrogen ions produced during anaerobic metabolism are buffered by intracellular buffers, including proteins, and by extracellular buffers, the most quantitatively important of which is bicarbonate. Despite this buffering, intense exercise induces a pronounced acidosis and acidemia with decreases in arterial and mixed venous pH and base excess, decreases in arterial bicarbonate concentration, and marked increases in carbon dioxide tension in venous blood. The acidosis associated with maximal exercise is severe and tolerable only for short periods of time. Resolution of the respiratory acidosis occurs within seconds to minutes of the cessation of exercise, whereas metabolic acidosis is slower to resolve, taking 30–60 minutes.

Muscle contraction produces heat which, if not effectively dissipated, results in hyperthermia (see Chapter 6.3). The heat generated by an exercising horse is sufficient to raise its body temperature by 3–5°C. If exercise is prolonged and not accompanied by effective heat dissipation, the rectal temperature may exceed 42°C, a temperature associated with markedly increased risk of heat shock and illness. Heat generated in muscle is transported by the blood to the skin and respiratory tract, from where it is lost into the ambient air. Heat dissipation from horses is achieved by evaporation of sweat, evaporation of respiratory tract secretions and convective loss of heat in air moving over the horse's skin and respiratory membranes.

Physiology of training

Training is essential for horses to compete effectively and safely. All equine athletes undergo some type of training regimen to prepare them for the rigors of competition. Training prepares the equine athlete for competition by inducing the physiologic adaptations necessary to perform at a high level with minimal risk of injury, and by providing the appropriate behavioral and psychological factors essential for effective competition. In order to prepare a horse adequately for competition, the horse should regularly perform the

type of activity that it will perform in competition at an intensity that will induce the physiologic changes needed to permit optimal performance.

Repetitive exercise induces a multitude of physiologic and anatomic adaptations in horses. The specific responses of each body system are dealt with in detail in the relevant sections of this book. However, there are a number of concepts that are common to many body systems.

The adaptive response

An important concept is that some physiologic processes, functions, or anatomic structures are malleable and able to adapt in response to the stresses and strains imposed by repetitive exercise. Collectively, induction of these adaptive responses to exercise is called training or conditioning. Strictly speaking, training refers to changes in behavior induced by certain practices whereas conditioning refers to the physical changes that occur in response to repetitive exercise. However, the terms are often used synonymously.

The adaptive responses induced by repetitive exercise act to reduce the effect of the strain induced by the physiologic stressors associated with exercise. The body acts to minimize the disruption to homeostasis induced by exercise by increasing the capacity of the system to deal with the work imposed by exercise. For example, the stress of increased force production by muscle during exercise stimulates changes in muscle structure and function that act to reduce the stress on individual muscle fibers, while increasing the overall capacity of the muscle. This phenomenon is common to many, but not all, body systems and the cumulative effect is a change in body composition and capacity for physical work.

Mechanisms of training effects

Repetitive exercise (exercise training) results in a multitude of changes in the body at cellular, tissue, organ, and whole organism levels. At the most fundamental level, training occurs through increased production of proteins, both structural and functional proteins. Accumulation of metabolites and waste products is believed to induce increased transcription of DNA specific for proteins, including enzymes, that control rate-limiting functions associated with these metabolites.

Increased transcription, if associated with increased translation of mRNA to protein and appropriate post-translational events, results in production of more protein. The increased quantity or activity of the enzymes then results in an increase in the maximal rate at which the metabolites can be processed or waste products eliminated. At an organ level these changes result in an increase in function, usually associated with increases in organ size. Interestingly, there are genetic determinants of the response to training in humans, with people with the D allele of the *ACE* gene having greater responses in heart size to training.^{33,34} The contribution of specific genes to training responses of horses has not been elucidated at the time of writing.

Principles of training

For training to be effective in inducing the desired conditioning, there must be a degree of 'over-reaching'. Over-reaching refers to the performance of an activity at a sufficient intensity and duration to induce some strain into the organism. Without this strain, there will be no conditioning effect.

It is also important to recognize that training is task-specific. The task for which conditioning is desired must be performed. For example, a horse trained to compete in endurance events will be poorly trained for sprint racing. Given the specificity of training, there are three principles of training expressed for human exercise physiology:³⁵

1. Repetition
2. Summation
3. Duration.

To induce a training effect, there must be repetition of the training stimulus. The number of repetitions varies with the type and intensity of exercise. Summation refers to the total amount of work performed. To achieve some degree of over-reaching, the total amount of work performed must be sufficient to induce some strain. If tasks are performed without sufficient time for substantial recovery between repetitions, then the total amount of work needed to achieve a training response may be lower than if recovery is allowed to occur.³⁵ Finally, the training stimulus must be of sufficient duration to induce an effect.

These principles of training must be used in a thoughtful and planned manner in order to induce the maximum training response while reducing the risk

of injury. The art of training involves the judicious use of exercise of various intensities and durations in order to induce the optimal adaptations that will permit successful competition while preventing injury or occurrence of overtraining.

Overtraining

Overtraining is defined as a loss of performance ability — despite the maintenance of or an increase in training effort — which cannot be explained by any discrete pathology. Overtraining is a well-recognized syndrome in human athletes in which increases or maintenance of training intensity are associated with decrements in performance.^{36,37} Diagnosis of overtraining in humans is complicated by the absence of any one definitive test, although psychological profiles, including evaluation of mood, are the most specific indicators of overtraining.³⁶ The situation is even more complicated in horses for which psychological and mood evaluation is not available. Overtraining in horses is characterized by decrements in performance and maximal rate of oxygen consumption.^{38,39}

The syndrome has been characterized by decreased performance capacity and loss of weight, as well as behavioral changes (irritability), including a reluctance to exercise.⁴⁰ The horses often are nervous, have tachycardia, muscle tremor, sweating, and diarrhea even before a race or in connection with training, and show inappetence. There is no single change in routine hematological or serum biochemical variables that provides confirmation of overtraining. Overtraining is an imbalance between training and recovery, exercise and exercise capacity, stress and stress tolerance. Improper nutrition may contribute to the overtraining syndrome. Chronic fatigue, lack of training progress, and injuries are common outcomes.⁴¹

The physiologic features of overtraining are still poorly understood. In addition to depletion of energy stores and damage to muscle cells, it probably involves endocrine imbalance. Hormones are essential for physiological reactions and adaptations during exercise and influence the recovery phase after exercise by modulating anabolic and catabolic processes. In overtrained human athletes, disorders of hormonal regulation at pituitary–hypothalamic level, adrenal exhaustion and a consequent reduction in blood cortisol, down-regulation of peripheral and perhaps central β -adrenergic receptors, and also down-regulation of neuromuscular junction have been

claimed to occur. Overtrained horses have either increased or decreased adrenocortical responsiveness to adrenocorticotrophic hormone (ACTH) administration.^{40,42}

Factors limiting performance

Maximal performance involves the coordinated optimal functioning of almost all body systems. In most cases, maximal performance requires that these body systems operate at or close to their maximum capacity. Conceptually, this integrated maximal function may be viewed as a pipeline. The maximum overall flow through the pipe is limited by the narrowest segment of the pipe. This analogy is often employed for oxygen transport during exercise and the system is viewed as one of tuned resistors, with no one individual element limiting the capacity of the system.⁴³ While this analogy is appropriate for healthy animals, it may not be so for animals with performance-limiting abnormalities, such as lameness or airway obstruction. In this instance, a single abnormality is sufficient to impair performance. Specific aspects of poor performance are dealt with elsewhere in this book.

For healthy horses the actual performance-limiting factor depends on the type of exercise and its duration. Standardbred or Thoroughbred race horses running at top speed are probably limited by oxygen transport. In these animals the malleable components of the oxygen transport chain (red cell mass, mitochondrial

volume, muscle capillarity) have adapted to the extent that the capacity of these components approaches or exceeds the capacity of the non-malleable components, such as lung volume or tracheal diameter. A reduction in the capacity of the non-malleable component — for example, a reduction in laryngeal diameter secondary to laryngeal hemiplegia — will reduce the capacity of the whole system. This has important consequences for a horse performing at maximal intensity. However, if the capacity of the non-malleable components exceeds that of the malleable components, then a reduction in capacity of the non-malleable component may not reduce performance: for instance, in the case of a dressage horse with laryngeal hemiplegia. In this case, the disorder will probably not limit the physiologic capacity of the horse to perform its task (although the associated respiratory noise may detract from the performance).

For other types of performance, other factors are limiting. Three-day event horses may be limited by their capacity to thermoregulate, endurance horses by their capacity to maintain fluid and electrolyte homeostasis, and draft horses by the strength of their muscles. Clearly, the factors limiting exercise capacity of horses vary with the type and duration of exercise. However, an understanding of what is likely to limit performance for each breed and use of horse is important not only in understanding the physiology of that form of exercise, but also in determining the likely causes of poor performance in animals with clinical disease.

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Exercise testing in the field

David Evans

Introduction

Exercise tests of equine athletes can be conducted in a treadmill laboratory or in the field. There are advantages and disadvantages for conduct of exercise tests in both these locations. Field investigations have the advantage of conduct of the test in the environment likely to be used in competition. The surface, gaits, and speeds used in a field test are therefore more closely aligned to the demands that horses face during exercise in the 'real world'. Field tests also account for the effects of jockey or driver. These advantages can also be disadvantages, because they can contribute to difficulties in standardization of field exercise tests. This chapter presents some of the limitations of treadmill exercise tests, and describes techniques that have been used for field exercise tests in galloping, trotting, and pacing horses. The rationale for making the effort to perform field exercise tests is discussed. There have been many remarkable field studies that have measured the electrocardiogram, collected arterial blood and measured tracheal pressures in galloping horses. However, it is not the intention of this chapter to review in detail all equine field studies. The scope of this chapter will be limited mostly to field studies of cardiorespiratory function, and to the use of field studies to conduct fitness tests in athletic horses.

Limitations of treadmill tests

The arguments for using high-speed treadmills to evaluate fitness and health of horses are obvious. The physical environment can be controlled, and conduct of exercise tests with precise design is possible. The speeds and durations of each step of an exercise test

are highly repeatable. There is also easy access to horses at suitable times during and after exercise for cardiorespiratory measurements and blood collections.

Horses should be acclimated to treadmill exercise before clinical exercise testing.¹ However, responses to acclimation runs are unpredictable in individual horses. Figure 1.2.1 illustrates the variability of heart rates and plasma lactate concentrations during treadmill exercise in horses that were given four treadmill tests on consecutive days. Several studies have shown that physiologic responses to treadmill exercise do not replicate responses to field exercise. Heart rate (HR) and plasma lactate concentrations in Standardbred horses pulling a 10 kilopond draught load were lower on the treadmill than on the racetrack.² It was also reported that HR and blood lactate in trotters were lower during exercise on a level treadmill than during exercise on a racetrack (Fig. 1.2.2).³ Heart rates were expressed as V200 and VHRmax, the velocities at which HRs were 200 beats per minute or had just reached maximal HR. Blood lactates were expressed as V4, the velocity at which blood lactate was 4 mmol/L. This value is sometimes referred to as VLa4. Stride frequency was lower and stride length was greater on the treadmill. Interestingly, this study also showed that there were no differences in any measurements on two sand tracks of 720 and 1250 m in length. A study of ridden Warmblood horses also found that HRs and blood lactate concentrations were lower on the level treadmill at speeds of 6.5–9.4 m/s compared with exercise over ground. The treadmill speed had to be increased by approximately 10% or the treadmill incline increased to 1–2% to give the same heart rates as in the field.⁴

Locomotion during treadmill exercise is quite different to that on the track. As a consequence, even if horses are given tasks on treadmills that produce

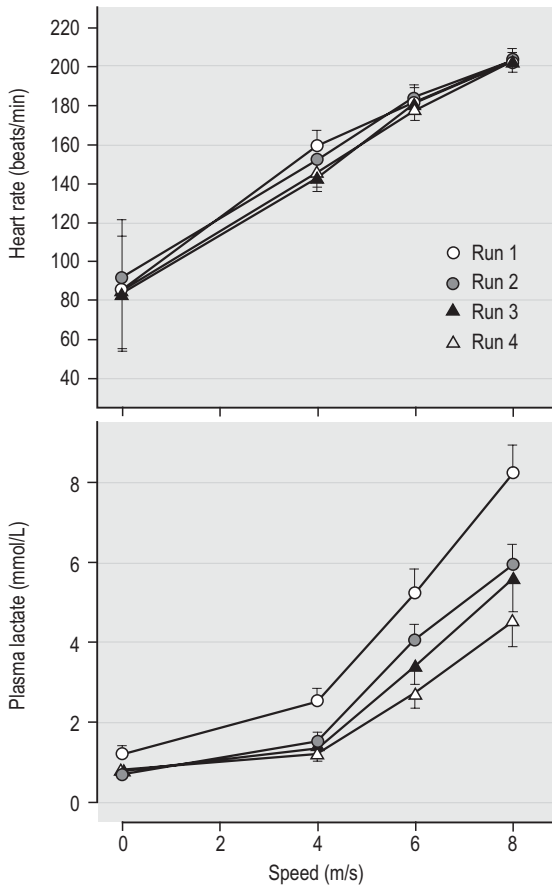


Fig. 1.2.1

Heart rates and plasma lactate concentrations (mean $\pm$ SEM) during treadmill exercise in six race horses that had four sequential acclimating runs over a 4-day period. Note the variability of heart rates during trotting at 4 m/s, and the high variability of plasma lactates at all velocities. (From King et al,¹ with permission.)

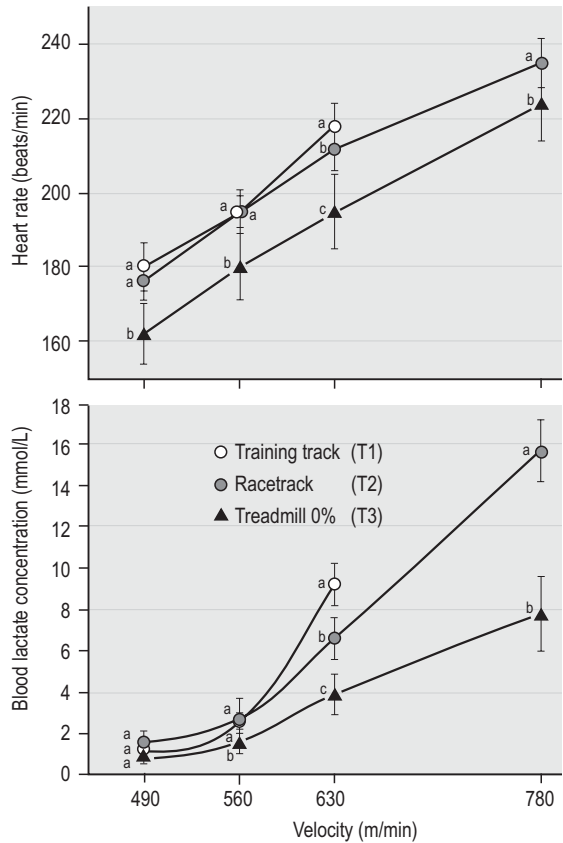


Fig. 1.2.2

Mean $\pm$ SD heart rate (HR) and blood lactate concentrations in five French Standardbred trotters at three different speeds during exercise tests performed on two different tracks (T1, T2) and on a non-inclined treadmill (T3). At each velocity, values with a different letter are significantly different. The treadmill tests produced lower HRs and blood lactate concentrations in most cases. (From Courouc   et al,³ with permission.)

similar HRs, their gaits may be quite dissimilar. Stride frequencies at identical trot and gallop speeds are greater on a racetrack.⁵ Design of treadmill exercise tests to replicate field exercise therefore seems to be a fruitless endeavor.

Treadmill exercise tests should be used when it is appropriate to do so, and field exercise tests also have a role in the management of athletic horses. In field tests, horses do not need acclimation, and the exercise is conducted in the physical environment that is more closely matched to that used in competition.

Field exercise tests

Heart rate and blood lactate measurements are the bases of an exercise test for athletic horses. Heart rates are usually expressed relative to a constant submaximal speed, such as V₂₀₀, the velocity at which heart rate is 200 beats per minute. However, expression of the velocity at lower heart rates is equally valid, and some studies have used V₁₄₀ and V₁₇₀.

The blood lactate response to specific speeds of exercise has also been used in numerous studies of field exercise testing for assessing performance and fitness. Fitness has usually been described with speed at a lactate concentration of 4 mmol/L (VLa₄). As the

horse increases fitness, VLa4 increases. Alternatively, the blood lactate response to a single episode of submaximal exercise can be used. This 'one-step' approach might be more applicable in field tests. It obviates the need for time-consuming, standardized multiple increments of velocity, and the need for multiple collections of blood via a catheter secured in the jugular vein or by repeated venipuncture. Treadmill exercise tests have also measured the lactate 'break-point', the velocity at which blood lactate begins to accumulate in the blood,⁶ but there has been no application of this technique in the field.

All exercise tests should attempt to answer a simple question for a trainer or owner of the horse. Ideally the exercise test should be designed to answer one or more of the following questions:

1. Has the horse's fitness changed with recent training?
2. Is the horse 'fit' for its next race, where fitness refers to a horse that is healthy and suitably trained?
3. How does the fitness of horse A compare with horse B?
4. Does a horse with poor racing performance have suboptimal fitness?
5. Can an appropriate measure of fitness help with training of race horses?
6. Does a horse with suboptimal or unexpectedly poor performance have a disease? Is there evidence of a cardiac or respiratory limitation to performance?

Exercise tests to help answer all of these questions necessitate measurements of heart rate, oxygen uptake, and pulmonary ventilation. Nevertheless, blood lactate and heart rate measurements during or after suitable exercise tests can help with answers to questions 1, 3, 4, and 5. In the following sections, field exercise tests that have used heart rate and blood lactate measurements in Standardbred and Thoroughbred horses will be described.

There are several important principles to follow so that field fitness tests provide meaningful results and answer one or more of the above questions. First, the test protocol should be simple. Multiple steps of increasing speeds of exercise are frequently used in treadmill testing, but these forms of exercise testing in the field may not be popular with trainers because of the excessive time commitment. Exercise tests should also be easy to implement, and ideally should not disrupt normal training schedules.

There are several features of the exercise test that should be maintained wherever possible. These include consistency of:

1. Warm-up routine prior to testing
2. Rates and distances of acceleration during the exercise
3. Test distances or times
4. Speed during the exercise
5. Time after exercise at which blood is collected
6. Post-exercise activity
7. Environmental conditions.

The environmental conditions can be an important factor during the conduct of field exercise tests.⁷ Heart rates and other variables were compared under high and low ambient temperature and relative humidity during a submaximal incremental field exercise test in horses tested in summer and in autumn. Heart rate was measured continuously, the other variables at rest and immediately after 4 minutes at 3.5, 4.5, and 7.0 m/s, separated by 3-minute rest intervals, and after 5 and 10 minutes recovery. Heart rates were significantly greater by a mean of 13 beats per minute during exercise in the hot versus cool conditions. It was concluded that differences in environmental conditions can affect assessment of exercise response. These factors must be considered when using fitness tests in the field. Sudden changes in environmental conditions might have considerable consequences for heart rates during exercise.

There has been slow adoption of the use of field exercise tests in commercial race-horse training establishments. Part of the reason for the slow adoption of these techniques has been the difficulty in the design and implementation of exercise tests in the field. Treadmills are useful because they help with the conduct of standardized exercise tests. However, few trainers use treadmills or have access to them. Understandably, some trainers might also have reservations about adopting new techniques that could disrupt busy training schedules.

However, many horse owners continue to be frustrated by lack of information about the fitness and performance capacity of their horses. Several recent studies have outlined new methods of performing exercise tests on racetracks. Some of these methods could easily be implemented in commercial training environments, so that they are a part of the routine management of the horses. The general approaches described below for use of heart rate and blood lactate measurements for fitness assessment can also be applied in endurance, event and other athletic horses. Ergospirometry, the measurement of breathing and oxygen uptake during exercise, is necessary for an

ideal exercise test, but the technology is not yet suitable for routine use in the field.

Studies of heart rate in galloping horses

During the 1960s and 1970s, before the common availability of high-speed treadmills at research centers, there were many field studies with remarkable achievements. Telemetric electrocardiography was used widely in the 1960s and 1970s to study the HR and electrocardiogram (ECG) of race horses during exercise on racetracks.^{8–13} Direct recording of the ECG with an on-board tape recorder was also used to study the heart rate during races.¹⁴ These studies described typical heart rates during submaximal and maximal exercise in Thoroughbreds and Standardbreds. Studies of training exercise and races were included, as were descriptions of the recovery of heart rate after field exercise. Studies of heart rate were also combined with telemetry of arterial blood pressure at speeds up to 800 m/min.¹⁵ These studies were mostly descriptive, and did not focus on design of exercise tests.

An exercise test typically consists of several bouts of exercise after a warm-up, which may or may not be separated by a rest period. Heart rate is usually measured during the exercise, and the velocity of each step of the exercise test is calculated by timing the event. The distances and durations of each step used in field tests have varied widely. Blood samples can be collected during rest periods after each step of the exercise test. Figure 1.2.3 shows a continuous record of heart rate over time during an exercise test in an Australian Thoroughbred event horse.¹⁶ In this exercise test, each horse was exercised over a 450 m distance at speeds of approximately 250, 300, 450, and 600 m/min. Horses were given a brief period of walking between each step of the exercise test. Figure 1.2.4 shows the use of a graph of heart rate and velocity for each step of the test to produce the typical linear relationship between heart rate and velocity. The graph also enables calculation of V200, the exercise velocity resulting in a heart rate of 200 beats per minute.¹⁶ In this study of 17 horses, V200 ranged from 560 to 900 m/min. This wide range could reflect differences in inherent fitness, and differences in fitness due to training. An increase in fitness results in an increase in V200. Loss of fitness, cardiovascular and respiratory disease, lameness, and an increase in bodyweight could all cause a decrease in V200.

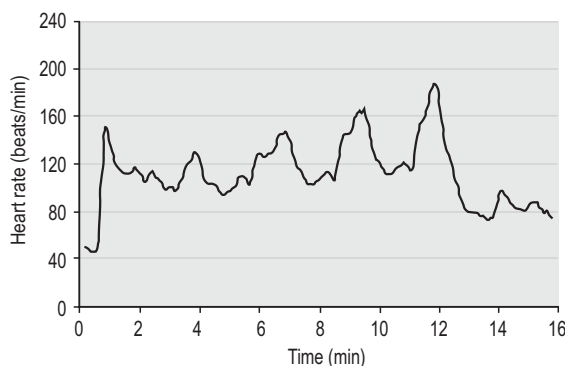


Fig. 1.2.3

Typical plot of heart rate versus time for the exercise test used in Thoroughbred event horses. There is an overshoot of heart rate at the commencement of the test (1 minute). The four heart rates during the four steps of the exercise test were recorded at 4, 7, 9, and 12 minutes. (From Serrano et al.,¹⁶ with permission.)

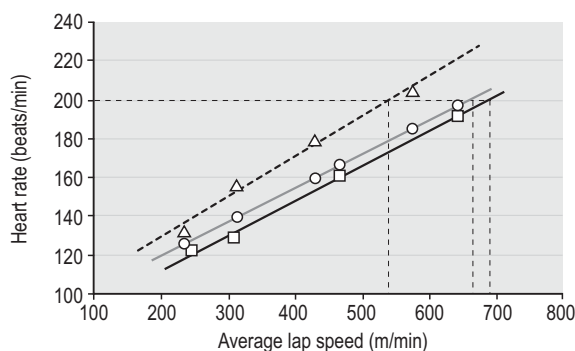
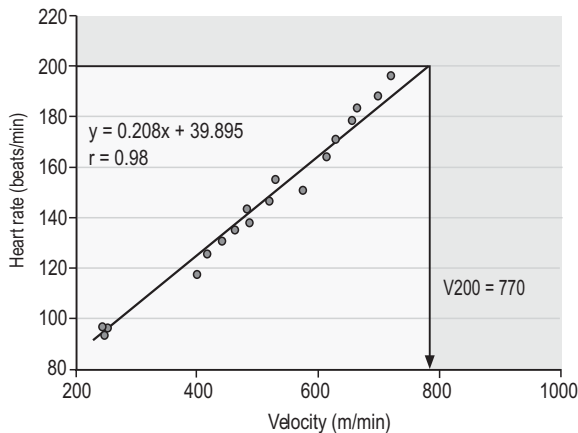


Fig. 1.2.4

Lines of best fit of the relationship between heart rate and velocity in a field exercise test used in two Thoroughbred event horses (△, □), compared with the average line of best fit (○) in 17 horses. Horse 2 (△) had higher heart rates than average at each of the four steps of the exercise test. The dotted lines show the method of calculating V200. (From Serrano et al.,¹⁶ with permission.)

V200 can also be calculated with an incremental field exercise test in Thoroughbred race horses.¹⁷ Commercial heart rate meters that log heart rate continuously and enable transfer of the data to a computer for analysis are suitable for this purpose. The exercise test consisted of about 1000 m trotting at 250 m/min, then galloping exercises at approximately 400, 460, 550, and 660 m/min for 600–800 m at each speed. Fine days and tracks in firm condition were used, and velocity was measured by stopwatch every 200 m of

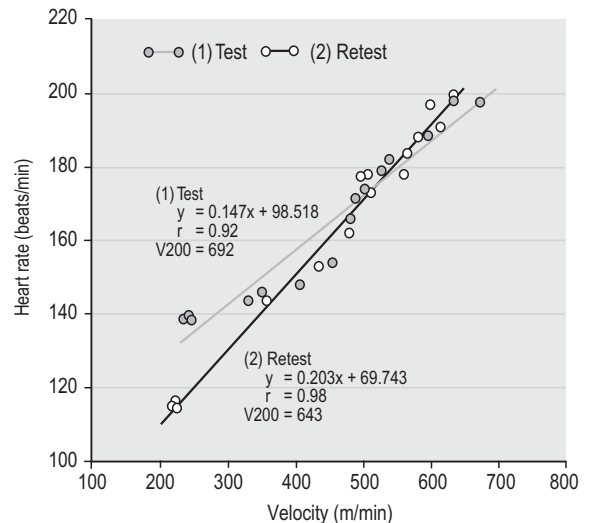
**Fig. 1.2.5**

Relationship between heart rate and velocity in a Thoroughbred race horse exercising under field conditions. (From Kobayashi et al,¹⁷ with permission.)

each step of the test. Mean HR and mean velocity were calculated for each 200 m section of the test.

This exercise test was used to investigate the influence of rider and track conditions, repeatability of V200 measurements, and the effects of training on V200.¹⁷ The HRs for the different sections of each step of the exercise test were all included, providing 17 HR and velocity measurements from one test. Figure 1.2.5 shows the relationship between heart rate and velocity in a Thoroughbred race horse using data obtained with this type of field exercise test. The method used in this exercise test has great potential. Use of a high number of data points should enable easy identification of outliers, and generation of a reliable line of best fit, as in Figure 1.2.5. The technique also shows that it is possible to generate excellent HR–velocity relationships in field tests without use of protocols that necessitate strict adherence to steps of an exercise test with constant exercise speed. Trainers may more readily adopt this technique because it does not necessitate changes to training schedules, and it can be incorporated into the usual daily training routine. The methodology in this study demonstrates the importance of refining field exercise tests so that they are easy to undertake. The usual treadmill model of an exercise test, with an emphasis on 45–60 seconds of constant velocity exercise in order to achieve steady state conditions, may not be the most suitable method in the field.

The importance of taking into account the psychological state of the horse was also demonstrated.¹⁷ In an excitable state, the slope of the regression line of HR

**Fig. 1.2.6**

Relationships between heart rate and velocity in a Thoroughbred race horse (1) exercising under field conditions after trotting in an excitable condition (●) and (2) when retested in a relaxed condition (○). Note the pronounced increase in heart rates when trotting at 250 m/min in an excited condition. This effect has a marked influence on the slope of the line of best fit, which causes a false increase in V200. (From Kobayashi et al,¹⁷ with permission.)

on velocity was decreased because of high HRs during trotting. V200 was thus falsely high. This finding emphasizes the need for careful observation of horses during field exercise tests, and for questioning of trainers and jockeys concerning the emotional state of the horse during exercise. If in doubt, calculation of V200 should be delayed until the horse has completed a test in a relaxed state. The correlation between 31 values for V200 measured on two consecutive days was 0.88.¹⁷ The differences in V200 were in the range of 0–50 m/min. Precision of V200 measurements in the field would probably be increased by use of more than one exercise test, and inspection of scatter plots to discard obvious outliers. These results suggest that the outliers will most likely be high HRs during trotting which could indicate excitability. Figure 1.2.6 shows the effect of excitability on heart rates during trotting, and the effect on V200. High HRs in this study were also associated with gait changes that were not 'smooth', and with phases of rapid acceleration. No major decisions about a horse's fitness or health should be based on the results of a single exercise test. Ideally, horses should be tested regularly, so that a record of results is established for an individual horse.

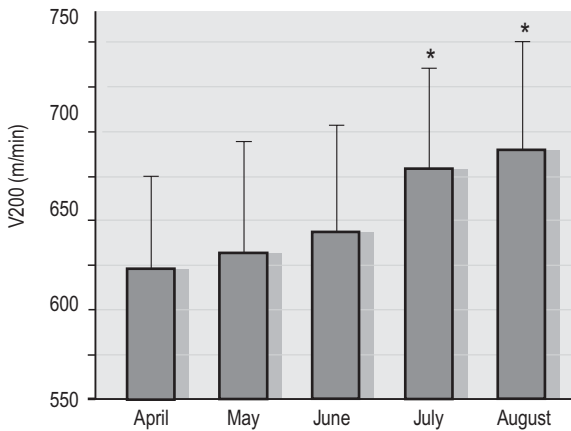


Fig. 1.2.7

Changes in V200 ($\pm$ SD) as training progresses from April to August in 2-year-old Thoroughbreds.

*Significantly different from April, May, and June. (From Kobayashi et al,¹⁷ with permission.)

If there is some doubt concerning the results in an individual exercise test, the test should be repeated.

As expected, the V200 was influenced by track type, with lower values on sand tracks than on grass or wood. Interestingly, the V200 was not significantly different in horses ridden by light (55 kg) and heavy (70 kg) jockeys in a crossover study.¹⁷ However, the mean V200 was 35 m/min higher with light jockeys, and it seems sensible to avoid large differences in field studies of ridden horses.

One important rationale for field exercise tests is to measure accurately, reliably, and precisely a variable or variables that indicate changes in state of training or health. Field studies of fitness have demonstrated that V200 in Thoroughbreds increased with training over a 5-month period (Fig. 1.2.7).¹⁷ In 2-year-old horses, the average increase in V200 over the period was approximately 65 m/min, an increase of approximately 10%.

Telemetric electrocardiography has been used in combination with pneumotachography to measure heart rate, respiratory frequency, tidal volume, and respiratory gas flow rates in four Thoroughbred horses during field exercise at a speed of approximately 800 m/min.¹⁸ Heart rate was also measured by telemetry during lunging exercise at the walk and trot, in combination with breath-by-breath measurements of pulmonary ventilation.¹⁹ Studies of minute ventilation, flow rates, respiratory times, and flow volume loops were conducted in normal horses and horses with airway diseases. Horses with bronchitis had

higher heart rates during exercise than normal horses, as well as altered measurements of pulmonary ventilation. The authors concluded that the technique had considerable potential for diagnosis and evaluation of therapies. However, there has been little adoption of this method because of the limitations of the technology for measuring breath-by-breath respiratory gas flow rates during field exercise.

The results of many studies have demonstrated that higher than expected heart rates during submaximal exercise may be an indication of one of the following:

1. Lameness, or another painful condition
2. Dehydration
3. Exercise conducted in hot conditions
4. A loss of fitness, due to detraining or inappropriate training
5. Respiratory disease
6. Cardiovascular disease, or anemia
7. Increased body mass, or a greater percentage of bodyweight as fat or water
8. A physiologically inferior horse, probably due to a relatively small heart.

Ideal use of exercise tests therefore depends on regular use of heart rate measurements during the training months. Conduct of one exercise test in isolation is much less likely to provide meaningful information. Comparison of current results with previous findings is most likely to give a trainer, veterinarian, or owner information that can help manage the horse's training program. As well, the finding of a high heart rate during an exercise test may or may not indicate a problem with fitness or health. High heart rates during exercise, compared with recent findings in the same horse, are not a diagnosis. However, they are a warning sign, and such horses should be thoroughly examined to ascertain whether or not there is a new clinical or other condition that could explain the results. It is also best to be cautious about interpretation of unexpected findings. Tests should always be repeated if possible to confirm the validity of results.

Field tests of fitness in Standardbred horses

Field tests with multiple speeds and blood collections have been conducted in Standardbred trotters and pacers to assess performance on the basis of HR and blood lactate measurements. A simple exercise test for pacing horses consisted of four steps of exercise over

1000 meters.²⁰ Speeds of each step were 450–550, 600–700, 700–800, and greater than 800 m/min. The horse walked for 3–5 minutes between each of the four steps. Blood was collected into fluoride oxalate tubes 3 minutes after each step. Heart rates were also recorded during the exercise test to calculate V200. A plot of speed versus blood lactate was drawn. By drawing a line horizontal to the 4 mmol/L concentration, the VLa4 can be directly calculated. It was observed that superior horses had a lower blood lactate response to this exercise test.

In a study of Swedish Standardbred trotters, 10 horses performed a similar submaximal test on a track. The test consisted of five incremental heats at approximate speeds of 9.1, 9.5, 10.0, 10.5, and 11.1 m/s over 1000 meters. A blood sample was drawn from the jugular vein for plasma lactate analysis immediately after each heat. The plasma lactate response to exercise differed between horses, but no correlation was seen with a racing performance index in a small number of horses.²¹

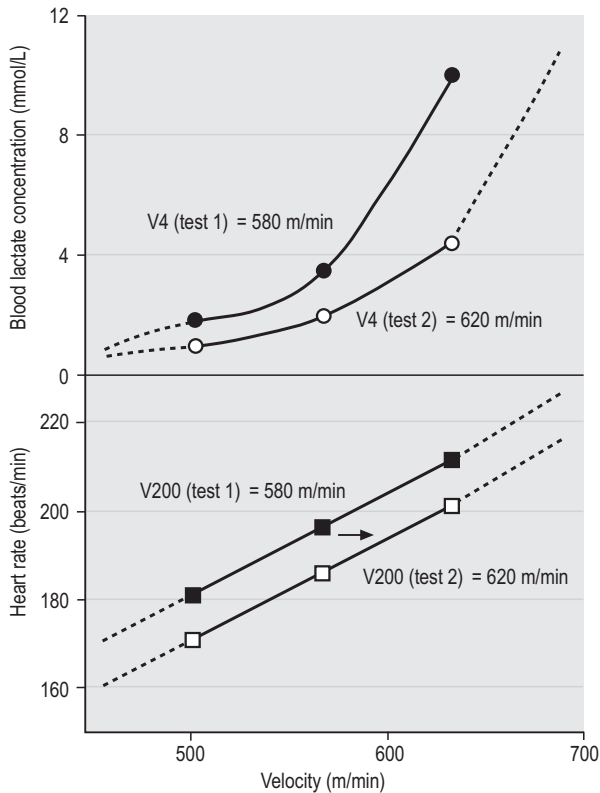
Studies of larger numbers of horses that have a large range of racing abilities are more reliable. The relationship between VLa4, age, and racing performance of Standardbred trotters has been investigated.²² A total of 159 horses performed standardized exercise tests of three steps performed at increasing speeds. The velocity of the horses was measured with a tachometer on the sulky. Mean VLa4 values increased significantly ($P < 0.05$) with age between 2 and 4 years. Horses were defined as good performers (GP) when finishing between first and fifth place in a race, or poor performers (PP) when finishing lower than fifth. VLa4 was significantly higher for GP than for PP ($P < 0.05$).

The VLa4 measurement is therefore a valid measurement for the evaluation of fitness in Standardbreds. The measurement could help trainers and owners to make more informed decisions about horses with poor performance, and assist with overall management of the racing career of a horse. Prospective owners may be more attracted to race-horse purchase if reliable measurements of fitness and performance capacity were more widely used. Veterinarians, trainers, or owners interested in using these tests in Standardbred horses should develop their own exercise test routine on a single racetrack. It should also be noted that blood lactate concentration is likely to be increased by excitement during the test, and by 'pulling', an inefficient gait due to effort expended against restraint by the driver. Results from exercise tests in which

horses pull hard against a jockey or driver should be regarded with suspicion, and the test repeated.

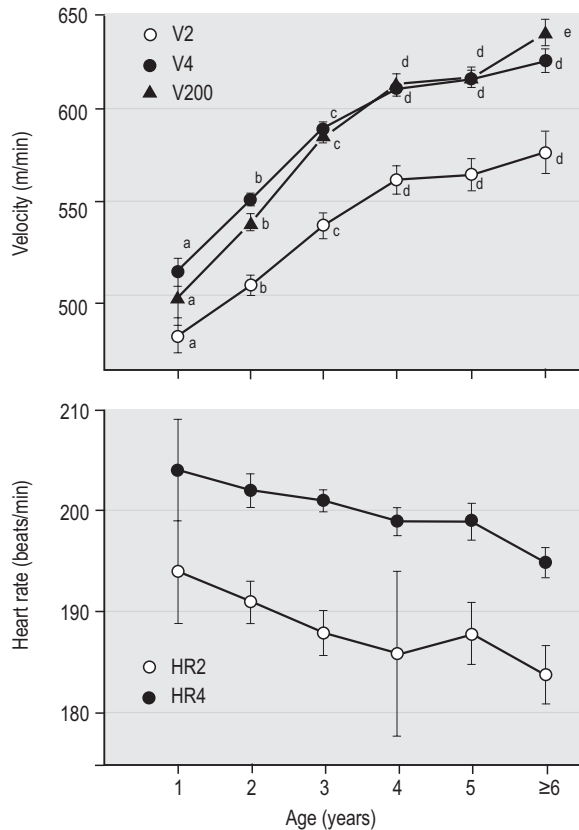
A review of exercise tests for French trotters exercising in the field concluded that track testing provided a more limited range of measurements than treadmill testing, but had the advantage of being performed in the horse's natural environment.²³ Various measurements such as heart rate during exercise and blood lactate concentration after exercise may be measured on the track, enabling calculation of physiological variables such as V200 and VLa4. Although VLa4 is calculated during submaximal intensity exercise, it is related to racing performance and seems to be the most important measurement to assess changes in fitness.²³ There is a significant influence of age on measurements of heart rate (V200) and VLa4 in trotters. Reference values for heart rate and blood lactate responses to field exercise in French trotters of varying fitness and age have been described.²⁴ The use of a graphical display of heart rate and blood lactate concentrations at different speeds of field exercise to calculate V200 and V4 in a trotting horse is illustrated in Figure 1.2.8.²³ This figure also shows the typical effect of training on the relationships; both curves shift to the right. Figure 1.2.9 shows the normal values for some heart rate and plasma lactate indices in relation to exercise velocity in trotters of various ages,²⁴ and the influence of training is described in Figure 1.2.10. High heart rates during field exercise tests may be associated with lameness or respiratory disease. The potential use of field exercise tests as an aid to the clinical evaluation of athletic horses is illustrated in Figure 1.2.11, which shows the effect of subclinical respiratory disease on heart rates and blood lactate concentrations during a submaximal field test in trotters.²³

A submaximal field exercise test consisting of two bouts of 1600 meters has been used to assess fitness in Standardbred pacing horses in two stables (A and B).²⁵ Five minutes of rest or walking between runs was allowed. Performance indices were compiled for each horse: number of race starts, number of race wins, number of race placings (1, 2, or 3), and lifetime earnings. Regression analysis was conducted to describe the relationship between plasma lactate concentrations and speed for tests one, two, and pooled results. Using the regression equation, observed (measured) minus expected (predicted) (O–E) lactate concentrations for tests were calculated and plotted against performance indices to determine their relationship. The association between lactate and velocity for the two

**Fig. 1.2.8**

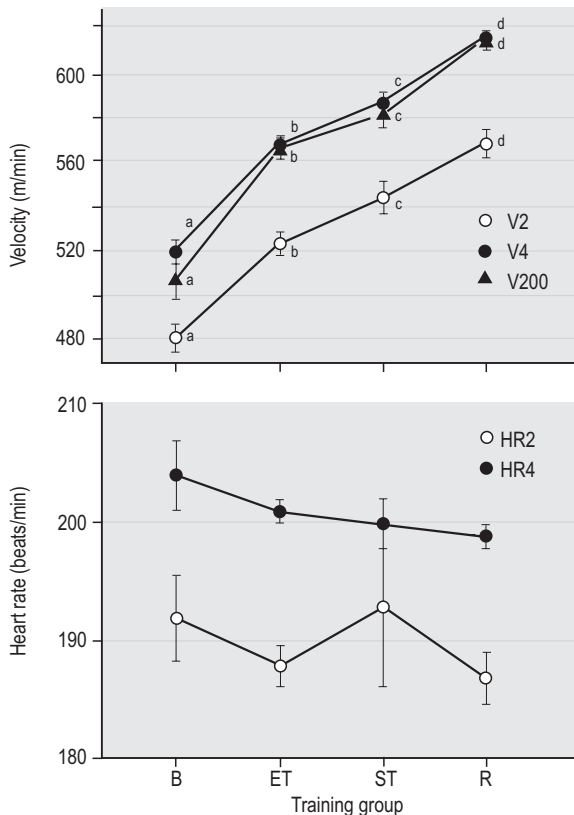
Heart rate and blood lactate concentrations related to velocity in a French trotter before (filled circles) and after (open circles) 6 weeks of training. There is a shift to the right with improved fitness. The heart rates and blood lactate concentrations during exercise at 500, 580, and 630 m/min are lower after training. V200 and V4 have increased. (From Couroucé,²³ with permission.)

tests was best described by exponential equations. This study found no relationship in either stable between O-E and performance indices (number of race wins, number of race placings, lifetime earnings, and average \$/start) for test run one, two, or pooled lactates. At one of the stables there was a significant association between V4 (velocity predicted to produce a blood lactate concentration of 4 mmol/L) and log lifetime earnings ($r = 0.51$, $P = 0.05$) and log average \$/start ($r = 0.54$, $P = 0.04$). There were no significant correlations at the other stable. It was concluded that a two-step determination method of V4 was a suitable method for studying limits to performance in pacing Standardbred race horses. A major advantage of the technique used in this study was that the test was easily incorporated into the normal training routines.

**Fig. 1.2.9**

Mean and 95% confidence interval values for several indices of fitness in French Standardbred trotters of various ages. V2 and V4, velocities at which post-exercise blood lactate concentrations are 2 or 4 mmol/L; V200, velocity at which HR is 200 beats/min. HR2 and HR4 refer to heart rates at blood lactate concentrations of 2 and 4 mmol/L. Values with different letters at each age are significantly different ($P < 0.05$). (From Couroucé et al.,²⁴ with permission.)

It was also noted that the correlations might be higher if studies of associations between fitness indices and performance included more horses with a wider range of racing abilities. Another limitation of such studies is that they do not include horses that have been discarded from training due to poor performance early in their racing career. More studies are needed of the variability in fitness of young, unraced horses. Does fitness in young unraced horses predict future racing performance? Is the rate of change in fitness equal in all horses when they are trained? Large-scale field studies may offer the best opportunities to investigate these questions.

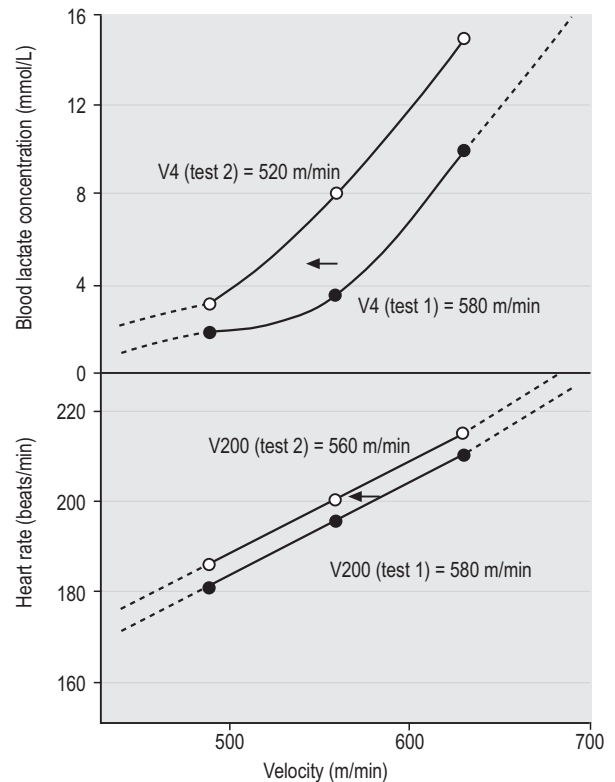
**Fig. 1.2.10**

Mean and 95% confidence interval values for several indices of fitness in French Standardbred trotters of various ages. B, at beginning of training; ET, after endurance training; ST, after sprint training; R, when racing. At each age, values with different letters are significantly different ($P < 0.05$). (From Couroucé et al,²⁴ with permission.)

Metabolic measurements after maximal exercise

Tests of the blood lactate response to submaximal exercise such as those described above are indications of endurance ability, or stamina. Lactate tests measuring VLa4 or VLa10 (blood lactate at a velocity of 10 m/s) are unlikely to be highly correlated with the ability of a horse to accelerate at the start or finish of a race, or the ability of a horse to sprint 600–800 m.

High levels of 'anaerobic stress' are found in Thoroughbred horses after approximately 50 seconds of maximal field exercise.²⁶ After only 400 meters of field exercise near racing speeds the blood lactate concen-

**Fig. 1.2.11**

Heart rate and blood lactate concentrations related to velocity in a French trotter before (test 1) and after (test 2) 6 weeks training, showing the influence of a subclinical respiratory infection. The heart rates and blood lactate concentrations during exercise at 500, 580, and 630 m/min are higher after training associated with the respiratory disease, and V200 and V4 have decreased. (From Couroucé,²³ with permission.)

tration increased from less than 1.0 mmol/L to over 14 mmol/L,²⁷ values similar to concentrations found after races. This rapid increase in blood lactate concentration during maximal exercise also occurs in Standardbreds and polo horses.²⁸ Measurement of the blood lactate concentration after maximal exercise has been used to estimate anaerobic capacity, defined as the ability of an individual to resynthesize ATP via anaerobic metabolism. Markers of anaerobic metabolism in skeletal muscle include concentrations of plasma lactate and uric acid after maximal exercise.²⁹

The blood lactate concentration after maximal exercise to fatigue does not change with submaximal treadmill exercise training,³⁰ or after high-intensity training.³¹ As well, the blood lactate concentrations

after maximal exercise were not correlated with race performance in trotters³² or Thoroughbreds.³³ This measurement is therefore not a useful marker of fitness.

Relationships between racing performance and plasma lactate and uric acid concentrations after racing were investigated in pacing Standardbred race horses.³⁴ Twenty horses were tested after races of 1760 meters and 28 horses after races over 2160 meters. Blood samples were taken 30–60 minutes before and 8 and 30 minutes after a race. There were no significant differences between the race distances for pre-race and 8 minute post-race plasma lactates. Significant low correlations were obtained for plasma lactate concentration 8 minutes post-race and the number of race wins ($r = 0.29$, $P = 0.04$), number of race placings (first, second or third), ($r = 0.34$, $P = 0.02$) and lifetime earnings ($r = 0.29$, $P = 0.04$). There were no significant correlations between performance indices and plasma uric acid concentrations in races of 1760 meters. For races of 2160 meters, correlations were found between plasma uric acid concentration at 8 minutes post-race and the number of race wins ($r = 0.37$, $P = 0.06$). As well, there was a significant correlation between uric acid concentration at 8 minutes post-race and lifetime earnings ($r = 0.35$, $P = 0.07$). These results imply that only 10–15% of the variability in retrospective career performance in pacing Standardbreds can be explained by these metabolic markers of the muscle anaerobic response to racing. Blood or plasma lactate and uric acid responses to maximal exercise are not useful measures of fitness on their own, but they could be included in multifactorial studies.

A study of the relationships between racing performance and several physiologic measurements was also conducted in 25 Standardbred trotters.²¹ Blood samples and muscle biopsies were obtained 5–10 minutes after racing. The biopsies were analyzed for fiber type composition and enzymatic profile and blood samples for plasma lactate and ammonia concentrations. Fiber type composition varied among horses (range 9–27% for type I, 32–54% for type IIA, and 27–46% for type IIB). Fiber type composition, muscle enzyme activities, plasma lactate, and ammonia responses to racing were not correlated to a racing performance index.

The rate of accumulation of lactate in blood during maximal field exercise over distances up to 400 meters is closely related to speed in Standardbred, Thoroughbred and polo horses.²⁸ It was suggested that this lactate measurement could be a useful index of

fitness. However, it is unlikely that any physiologic measurement after brief maximal-intensity exercise to estimate anaerobic capacity will be more closely correlated with fitness than a simple measurement of maximal speed during 40–50 seconds of exercise. In conclusion, the blood lactate response to maximal exercise has limited usefulness as a measure of fitness in horses. However, the blood lactate response to moderate or submaximal speed exercise, expressed as VLa4 or another similar index, is a useful technique for differentiating poor performers and good performers, and for monitoring the changes in fitness during training programs.

Blood lactate measurements in submaximal field tests of fitness in Thoroughbreds

A major difficulty with field exercise tests has been control of the exercise performed by the horse. Ideal standardized tests necessitate control of exercise speeds, duration of exercise, and rates of acceleration. Use of stepwise exercise tests has been usual in treadmill studies, with incremental speeds used. Such tests enable descriptions of the relationships between speed and variables such as heart rate, blood lactate concentration, and oxygen consumption. Field exercise tests of this sort are not very practical for Thoroughbred race horses, and alternative methods are needed. Conduct of racetrack exercise tests for measurement of VLa4 or VLa10 is especially problematic in Thoroughbreds because it is difficult to obtain constant track conditions and constant speeds during exercise. Measurement of the lactate responses to a single or pair of exercise bouts could be a superior approach to field exercise testing in Thoroughbreds.

A standardized, two-step exercise test has been used to investigate the blood lactate running speed relationship in nine Thoroughbred race horses.³⁵ Each horse completed a two-speed field test at intervals of 6–8 weeks to determine the running velocity (v) that resulted in blood lactate concentrations of 4 ($v(4)$) and 12 mmol/L ($v(12)$). Changes of $v(4)$ and $v(12)$ in a horse between two consecutive tests were used to assess the effects of training history. The percentage of days with gallop workouts between two consecutive tests showed a significant correlation with changes in $v(4)$ ($r = 0.71$, $P < 0.01$) and $v(12)$ ($r = 0.56$, $P < 0.05$). The number of gallop workouts ($r = 0.60$, $P < 0.05$) and the total time of training ($r = 0.58$, $P < 0.05$) also

correlated with the change of $v(4)$. Furthermore, the percentage of days without training was negatively correlated to changes of $v(4)$ ($r = -0.75$, $P < 0.01$) and $v(12)$ ($r = -0.56$, $P < 0.05$). These results imply that increases in fitness, as measured with the blood lactate response to submaximal exercise in a two-step field test, are more likely in Thoroughbred horses that have more galloping than trotting exercise, and have a higher number of gallops in a time period. More days without training was associated with reduced fitness, and more training at higher speeds was associated with greater fitness.

An alternative approach is to determine the blood lactate concentration during a single bout of strenuous, submaximal exercise. An appropriate speed of exercise must be chosen. The aim is to have an exercise test that is demanding for some horses, but achieved easily in others. Typical speeds for such tests are 800 meters in 65–70 seconds in a Standardbred horse,²⁰ and in 55–60 seconds in Thoroughbreds.³⁶ These speeds would need to be confirmed in an individual stable, because they will depend on the quality of horses being trained, and possibly on track size and surface conditions.

The use of a single blood lactate measurement after exercise has been validated as a measure of racing ability in Thoroughbred horses, and as a simple method of monitoring responses to treadmill training. A correlation of over 0.6 was found between retrospective career racing performance in Thoroughbreds, and blood lactate concentration 2 minutes after treadmill exercise at 10 m/s.³³ During a treadmill training program, the blood lactate concentration after treadmill exercise at 9 m/s gradually decreased over 9 weeks of training.³⁰ This response was similar to changes in VL₄. These results suggest that exercise tests with multiple steps may not be absolutely necessary for fitness evaluation in horses, and that measurement of the blood lactate concentration after a standardized, one-step test may suffice.

The feasibility of a one-step field test for assessment of fitness in Thoroughbred horses has been investigated.³⁶ Each horse completed a 1000 m warm-up at a slow trot of approximately 3–4 m/s prior to each test. Subsequently the horses completed an 800 m gallop with jockeys instructed to maintain a constant running speed in the range of 13–16 m/s. This range of speeds was selected as they correspond with the speeds frequently used in training Thoroughbreds on Australian tracks. The time for the 800 m and each of the four 200 m sections was obtained by stopwatch. All timing was conducted from the same position at

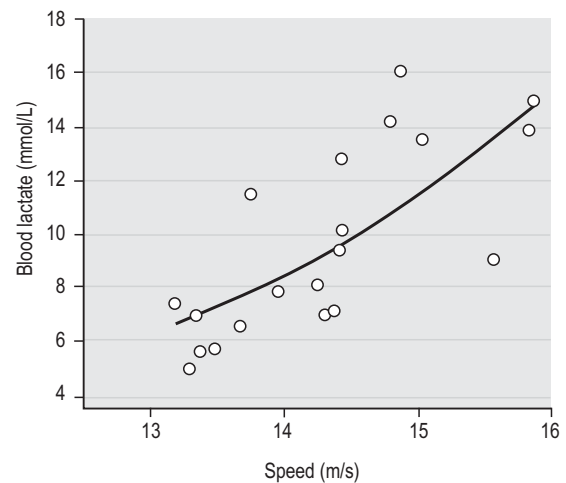


Fig. 1.2.12

Relationship between blood lactate concentration (mmol/L) after field exercise and velocity (m/s) during an 800 m exercise test on a sand racetrack in Thoroughbred horses. (From Davie & Evans,³⁶ with permission.)

each track by the same observer. Speed was determined from the total time for the 800 m gallop. At the completion of each gallop, the horse was trotted for 5 min, and then jugular venous blood was collected for blood lactate assays.

After inspection of the scatter plots of the relationships between blood lactate concentration and velocity for data from each racetrack, regression analyses were conducted to describe the line of best fit. Figure 1.2.12 shows the relationship between blood lactate after field exercise and exercise velocity on a sand racetrack in 21 trained Thoroughbred race horses.³⁶ The variability of the velocity during the exercise tests was expressed as the coefficient of variation (CV) of the times for the four 200 m sections of the exercise test. In horses tested more than once the result from the exercise test with the lowest CV was used in the regression analysis. Exercise tests were conducted at velocities in the range 13.2–15.9 m/s, and resulted in post-exercise blood lactate concentrations in the range 5.0–16.1 mmol/L. The mean speeds of the four consecutive 200 m sections of the exercise tests on the sand racetrack were 13.5, 14.6, 14.5, and 14.9 m/s. The jockeys were clearly unable to maintain a constant speed during the 800 m test.

A single-step test that takes into account variability of velocity within a test, and is based on calculation of

the difference between the measured and predicted lactate concentration, has potential application in field evaluations of fitness in Thoroughbred horses. Further studies are required to investigate whether the difference between the blood lactate response to exercise in an individual horse and the predicted concentration in a reference population is an accurate and reliable correlate of racing performance. Changes in this difference could also reflect changes in fitness over time.

The use of the difference between the measured and predicted lactate concentration as an index of the lactate response to exercise obviates the need for strict control over the target speed during the conduct of a field exercise test. Potentially it could be possible to conduct tests at any speed in an appropriate range, and compare the measured lactate concentration with the predicted concentration based on the equation for the line of best fit. This approach enables horses to be tested at different speeds, and comparisons between individual responses to exercise can be based on the rating. Superior English Thoroughbred race horses had a low blood lactate response to a single bout of exercise.³³ Therefore it could be expected that horses that consistently give a high 'rating' (that is, a high positive difference between measured and predicted lactate concentration) could be expected to race poorly. Superior training in an individual horse should reduce the difference between measured and expected blood lactate concentrations.

The validity of results could be improved with some refinement of the technique. Accuracy and reliability of measurements could be improved if velocity did not increase during the exercise test. Anxiety or fear could also contribute to the variance, and these factors may not always be easy to recognize. The confounding effect of increases in speed may be more important in field tests that measure blood lactate concentration than in tests that measure heart rate, because the increase in speed can be related to the higher heart rate when the data are analyzed, as demonstrated in a study with Thoroughbred horses.¹⁷

A simple field exercise test for event horses has been described.³⁷ In this test, horses warmed up with 5 minutes of walking and then 6 minutes of trotting. Horses then galloped 400, 500, 600 and 700 m/min over 1000 m with 5 min walking between these steps. This format enabled measurement of heart rates and blood lactate concentrations at each velocity of the exercise test, and calculation of V200 and VLa4. Such a test also assists horse trainers because it enables calculation of the velocity needed to train

horses at a predetermined heart rate or blood lactate concentration.

A field exercise test with three steps was used with eight French Thoroughbred horses in France to investigate the use of heart rate measurements during and after track exercise as a suitable measure of changes in fitness.³⁸ The test consisted of a warm-up followed by three 3 min steps, one cantering and two galloping, followed by a recovery period. Heart rate was recorded during the entire test, and blood samples were taken during the 2 min rest periods following each step, and after the recovery period for the measurement of lactate concentrations. Fitness was described by the relationships between lactate concentrations, heart rate, and velocity. The authors concluded that the efficiency score and the cardiac recovery index were good indicators of potential speed.

Total red cell volume measurements

Total red cell volume expresses the volume of erythrocytes in the circulation of the horse, including the volume in the spleen. Its measurement with dye dilution methods necessitates measurement of plasma volume and the induction of splenic contraction before the hematocrit (packed cell volume, PCV) is measured, so that red cells sequestered in the spleen at rest are also measured. Splenic contraction has been induced by epinephrine (adrenaline) injections and moderately intense exercise.³⁹ Total red cell volume relative to bodyweight was significantly correlated with maximal trotting speed over 1000 m in 35 Swedish trotters ($r = 0.68$, $P < 0.001$).⁴⁰ These results suggest that this measurement is an important factor in the ability to trot rapidly, but there have been no studies of the relationship in other breeds. Unfortunately measurement of the total red cell volume in horses with a dye dilution technique is not a simple procedure that can be readily applied in veterinary practice.

The hematocrit after maximal exercise in Thoroughbreds, which ranges from 60 to 70%, was not correlated with Timeform rating (a commercial measurement of relative racing ability) in English Thoroughbreds. It is not a valid fitness measurement.³³ However, the hematocrit can be measured after maximal exercise if results of resting hematology suggest that a horse is anemic. PCV should be greater

than approximately 55% immediately after maximal exercise in trained Thoroughbreds. In Standardbred race horses overtraining was associated with a decrease in PCV measured after a 2400 meter time trial at maximal speed. Mean values were 56% in control horses and 52% in overtrained horses.⁴¹ Total red cell volume did not decrease during overtraining, so the decrease in PCV may have reflected the decrease in velocity of the horses during the time trial, rather than a true decrease in the total red cell volume. A treadmill study has also found that onset of overtraining was not associated with red cell hypervolemia.⁴²

Measurements of the hormonal response to intense exercise may be more useful for identifying the overtrained horse. Overtraining was associated with a decrease in the cortisol concentration measured after a maximal field exercise test in Standardbred pacers.⁴¹ The exercise test consisted of 1200 meters pacing in 105 seconds, and then completion of the following 1200 meters in the fastest time possible. A decreased cortisol response to maximal exercise in the overtrained state was also found in a treadmill study of Standardbred horses.⁴³ The mean peak cortisol concentrations after intense treadmill exercise were 320 nmol/L before overtraining, and had decreased to 245 nmol/L when horses were overtrained. Overtraining should be suspected in horses with evidence of decreased performance in association with decreased bodyweight and plasma cortisol response to a standardized maximal or near-maximal velocity exercise.

Measurement of oxygen uptake in field exercise

Oxygen uptake is a fundamental measurement in any exercise test. It describes the rate of oxygen use in liters per minute, and is usually expressed relative to body mass. Calculation of oxygen uptake in the field necessitates measurements of air flow rates during breathing, and of oxygen and carbon dioxide concentrations in expired respiratory gas. The horse must wear a mask over its nose or face to enable these measurements. The technique is referred to as ergospirometry. In horses during maximal exercise, respiratory rates often exceed 120 per minute, and over 1500 liters of air are breathed per minute, at peak flow rates of

30–40 L/s or more at each nostril. Measurement of breathing (minute ventilation) and expired gas concentrations during intense exercise in horses is obviously a considerable technical problem, especially in the field.

Rates of oxygen uptake during submaximal exercise will depend on gait, economy of locomotion, body mass, and other factors. The maximal rate of oxygen uptake is the gold standard measurement for aerobic capacity. It is primarily limited by the maximal heart rate and cardiac stroke volume during exercise. A cardiac limit to performance can be best evaluated by measurement of oxygen pulse, the volume of oxygen ejected with each ventricular contraction. This measurement necessitates simultaneous measurement of oxygen uptake and heart rate during exercise.

A landmark study of heart rate, breathing, and oxygen uptake in two trotters was conducted in Russia.⁴⁴ A truck carried equipment beside the trotting horses, which completed a stepwise exercise test with peak speeds of 11 m/s. Notable findings during exercise at 11 m/s were tidal volumes of 17 liters, pulmonary ventilation of 1200 L/min, and peak oxygen uptake of 64 L/min with a respiratory exchange ratio of 1.0.

The horse plus vehicle approach to field ergometry was also used in a study of four Quarter horses, one Appaloosa and one Thoroughbred at speeds ranging from 40 to 390 m/min, with and without a rider.⁴⁵ A tractor was used to pull a wagon, and on the wagon were the calorimeter and a gasoline generator. This study was conducted to enable calculation of the digestible energy intake needed to support the demands of exercise.

Field ergospirometry was described in 23 riding horses at a walk, trot, and gallop, using an on-board oxygen sensor.⁴⁶ The key measurements of an ideal clinical exercise test were reported: heart rate, oxygen uptake, pulmonary ventilation, ventilatory equivalent for oxygen, oxygen pulse, and economy of locomotion. The synchrony of stride and locomotion was noted, as was the transitory effect of swallowing on breathing. The limitation of the performance of the pneumotachometer and response times of the oxygen sensor probably precluded measurements at maximal speeds.

Field ergospirometry and blood lactate measurements were conducted in 12 Warmbloods in order to calculate the ratios of aerobic and anaerobic contributions to total energy output at speeds up to approximately 500 m/min.⁴⁷ Aliquots of expired gas were

collected via tubes in the face mask, and the rider manipulated the bags that were used to collect the gas at each step of the exercise test. Oxygen debt was calculated from the oxygen uptake measurements made for 10 minutes after exercise, minus values before exercise. This value was referred to as the anaerobic contribution to energy output. It was reported that the percentages of energy expenditure that were anaerobic were 1%, 3%, 19%, and 30% at speeds of 100, 250, 350, and 530 m/min. This technology has not yet reported measurement of maximal oxygen uptake.

Breath-by-breath pneumotachography for clinical appraisal during field tests of ridden horses has made little progress in the last 20 years. A major technical challenge remains: specifically, simultaneous measurement of heart rate, oxygen uptake, and pulmonary ventilation during submaximal and maximal field exercise. Coupling of measurements of heart rate, breathing (pulmonary ventilation), and oxygen uptake during field exercise will be a powerful technique for advancing knowledge in equine exercise physiology.

Tracheal stethoscopy

The use of tracheal stethoscopy in the field for the investigation of respiratory sounds in the horse has been described.⁴⁸ This technique has been used for the investigation of upper respiratory conditions such as idiopathic laryngeal hemiplegia. The technique has existed for many years, but has yet to find a place in routine clinical exercise testing in the field.

Conclusion

Heart rate and blood lactate measurements during standardized field exercise tests are relevant to the management of all athletic horses. These measurements can assist with performance prediction and evaluation of fitness changes, and can be used to alert owners and trainers to problems such as lameness and respiratory disease. More effort is needed to adapt new technologies and refine approaches to design of field exercise tests. It is unlikely that veterinarians, trainers, or owners will be enthusiastic about equine fitness testing if the focus is not on simple approaches to field

exercise tests. Simple tests, measuring the things that matter, constitute the approach in field studies in human sports laboratories. Heart rate and blood lactate measurements feature prominently in human field studies. Progress in technology transfer of applied exercise physiology might be greater if there was greater emphasis on field methods, using minimally invasive techniques. Every fitness test should answer a specific question, and results should be expressed in a way that helps a veterinarian, trainer, or owner make more informed decisions about the training, fitness, health, or management of horses.

A promising new technique for field fitness tests could be use of heart rate measurements in combination with measurement of velocity with differential global position system technology. Simultaneous logging of a horse's heart rate and velocity could be a powerful technique for field exercise tests.^{49–51}

The reliance on treadmills for most equine exercise research may have contributed to poor rates of technology transfer to equine veterinarians, trainers, and horse owners. There are few established equine performance laboratories in the world, and many are located at considerable distances from racing populations. There will never be enough university-based treadmills to service all horses with sufficient facilities for fitness tests. Development of simple and user-friendly techniques for exercise studies of horses that do not depend on treadmills would therefore be a major advance.

New partnerships between equine exercise scientists and biomedical engineers could also generate new technologies for field studies. For example, field studies of breath-by-breath respiratory gas flows, and field ergometry, should be possible, building on the innovative studies performed in Germany.^{18,46} The techniques for field ergometry have been developed for human athletes, and could be refined for use in horses. However, the technical challenge of reliably and accurately measuring respiratory flow rates of over 100 L/s in a horse galloping in the field at 1000 m/min has yet to be conquered. Field ergometry, coupled with measurements of heart rate, respiratory function, and metabolic responses to exercise, would enable new fundamental studies in many areas of equine exercise physiology. Descriptions of the metabolic and energetic demands of different athletic events would be possible, and design of appropriate training programs would be facilitated. As well, clinical exercise testing would be more likely to be adopted by trainers and owners. Such 'high tech' clinical exercise tests would

contribute to greater knowledge concerning limits to performance in different events (such as anaerobic or aerobic capacity, and maximal rates of oxygen uptake). Greater rates of technology transfer to industry par-

ticipants are likely if researchers increase their use of normal horses in commercial training, and if technical developments free researchers from the constraints and limitations of treadmill fitness tests.

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Muscle physiology: responses to exercise and training

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Overview

A horse's skeletal musculature is highly developed, particularly in athletic breeds. In contrast to most mammals, in which 30–40% of bodyweight consists of muscle, or in non-athletic horse breeds (~42%), more than half (~55%) of a mature Thoroughbred's bodyweight comprises skeletal muscle.¹ Low body fat and a large amount of muscle likely reflect adaptation and selective breeding of animals used for elite endurance and sprint racing.² Total muscle blood flow in horses that are exercising at a level when $\dot{V}O_{2\max}$ 134 ± 2 mL/min/kg has been estimated at 226 L/min, which represents approximately 78% of total cardiac output.³ This exercise requires the integration of many different body systems under the control of the nervous system (Fig. 2.1.1). Metabolites and oxygen reach skeletal muscle fibers via the respiratory, cardiovascular, and hematologic systems; in turn muscle fibers produce energy in the form of adenosine triphosphate (ATP), which, via the contractile machinery, is converted into mechanical work. The structural arrangement of the musculoskeletal system provides the means with which to harness this energy, either moving the horse's limbs in a characteristic rhythmic pattern for each gait, or enabling diaphragm contraction, which contributes substantially to inspiratory effort.⁴

Equine muscle is considerably heterogeneous, the diversity reflecting functional specialization and adaptive plasticity that has been studied extensively over the past 30 years. Muscle biopsy in particular has resulted in a greater understanding of the response of this tissue to exercise and training, and much of this work is summarized in the excellent review by Snow & Valberg.⁵ This chapter focuses specifically on new data obtained in the past decade, while assessing earlier studies from a later perspective.

Methodology

Percutaneous needle biopsy technique

Equine muscle physiology has centered on use of percutaneous needle biopsy (Fig. 2.1.2),^{6,7} a technique originally described for the M. gluteus medius by Lindholm & Piehl.⁸ This is the heaviest muscle of the entire pelvic limb;⁹ it is very active during exercise¹⁰ and shows considerable adaptation to training.⁵ However, care must be exercised in interpreting data from a single biopsy from this muscle,¹¹ particularly given its heterogeneity.^{12–14} Other locomotory muscles (e.g. semitendinosus, biceps femoris, longissimus lumborum, triceps brachii and cleidocephalicus) can also easily be biopsied using the same technique.

Muscle samples are useful for studies *in vivo* and *in vitro* using a range of morphologic, biochemical, and physiologic techniques. Muscle samples for histochemistry are frozen in isopentane pre-cooled in liquid nitrogen. Samples for biochemistry are immersed directly in liquid nitrogen. In addition, a portion of the sample may be directly fixed for electron microscopy, thereby allowing analysis of ultrastructure and measurement of capillary¹⁵ and mitochondrial density.¹⁶ Biochemical and physiologic studies can also be made on dissected and skinned single fibers.^{17–19}

Laboratory methods

Biochemical analysis of homogenized equine muscle samples has enabled the study of broad metabolic responses to exercise and metabolic adaptation to training (see reference⁵ for a review) through analysis of selected muscle enzyme activities, their substrates, and intermediary metabolites. However, homogenization of samples prevents the differentiation of the various individual fiber types or the study of important

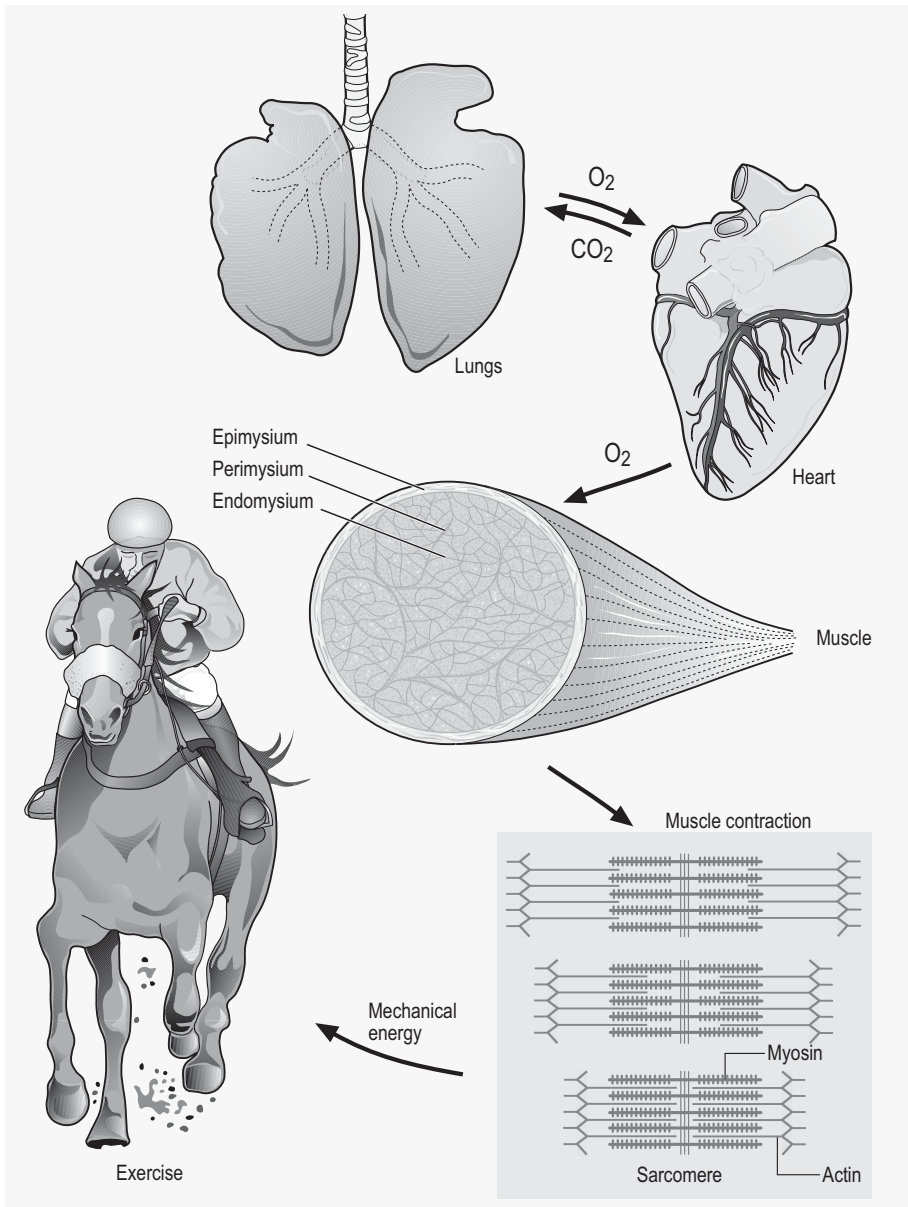


Fig. 2.1.1

Interaction between the main body systems (respiratory, cardiovascular, hematologic and muscular) involved in exercise.

morphologic features such as fiber size, capillary density, and myonuclear location. Some of these disadvantages can be overcome by analyzing single fibers biochemically.^{20,21} Histochemical evaluation of muscle, combined with image analysis,²² has provided invaluable information about the contractile and metabolic properties of equine muscles, specifically

regarding fiber types, oxidative and glycolytic capacities, fiber sizes, and capillary density. However, subjective visual assessment of qualitative histochemical reactions (Fig. 2.1.3A,B) has until recently limited the application of these methods.²³ In recent years, more objective and quantitative histochemical methods have been applied to equine muscle.²⁴

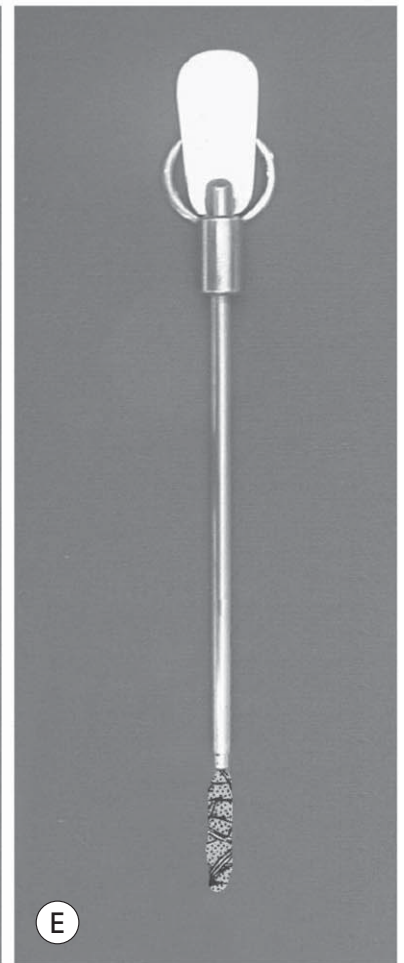
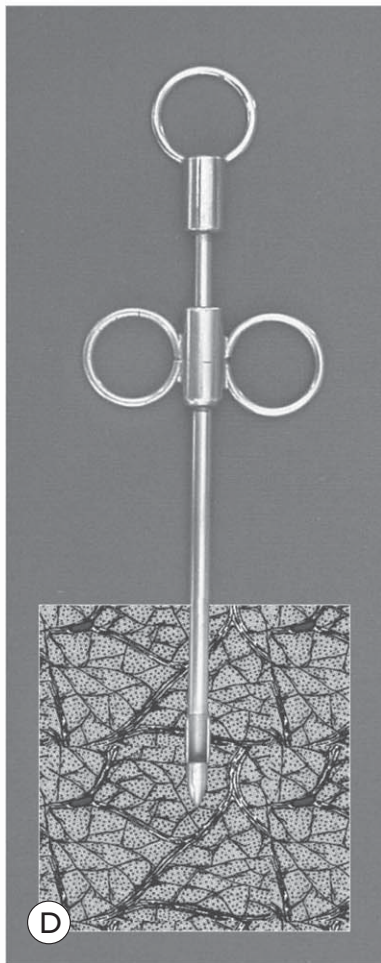
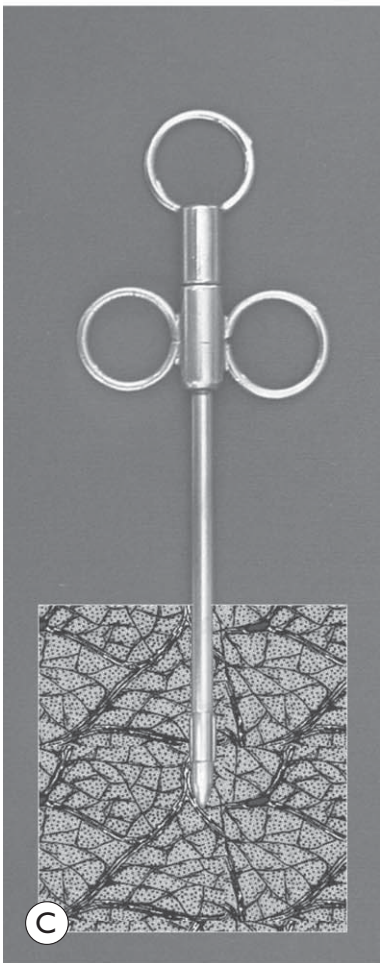
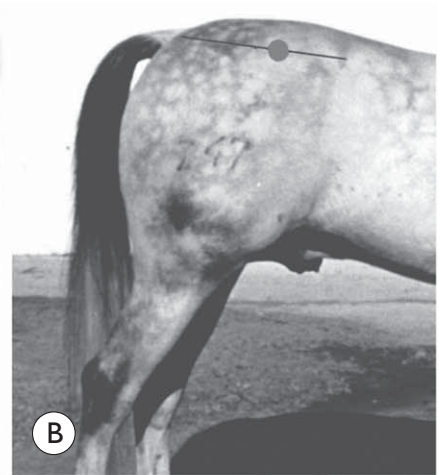
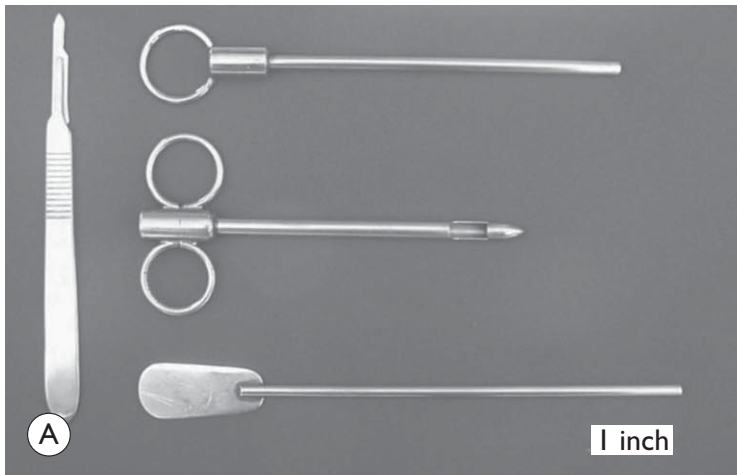


Fig. 2.1.2

(A) Percutaneous muscle biopsy needle; this needle, which has an outer diameter of 6 mm, was first introduced by Bergström⁶ and was further modified with finger and thumb rings by Henckel.⁷ (B) Site for the collection of biopsy specimens from the right gluteus medius muscle according to Lindholm & Piehl;⁸ this fixed site is located one-third of the distance along a line running from the tuber coxae to the root of the tail. (C–E) An illustration of the various steps for the needle biopsy technique; (C) the needle biopsy, together with the internal cutting cylinder, is inserted into the muscle; (D) once within the muscle, the cutting cylinder is partially withdrawn so that the window is opened up, allowing muscle to enter the slot, and a piece is then cut by pushing down the internal cylinder; (E) between 50 and 150 mg of muscle tissue are usually acquired.

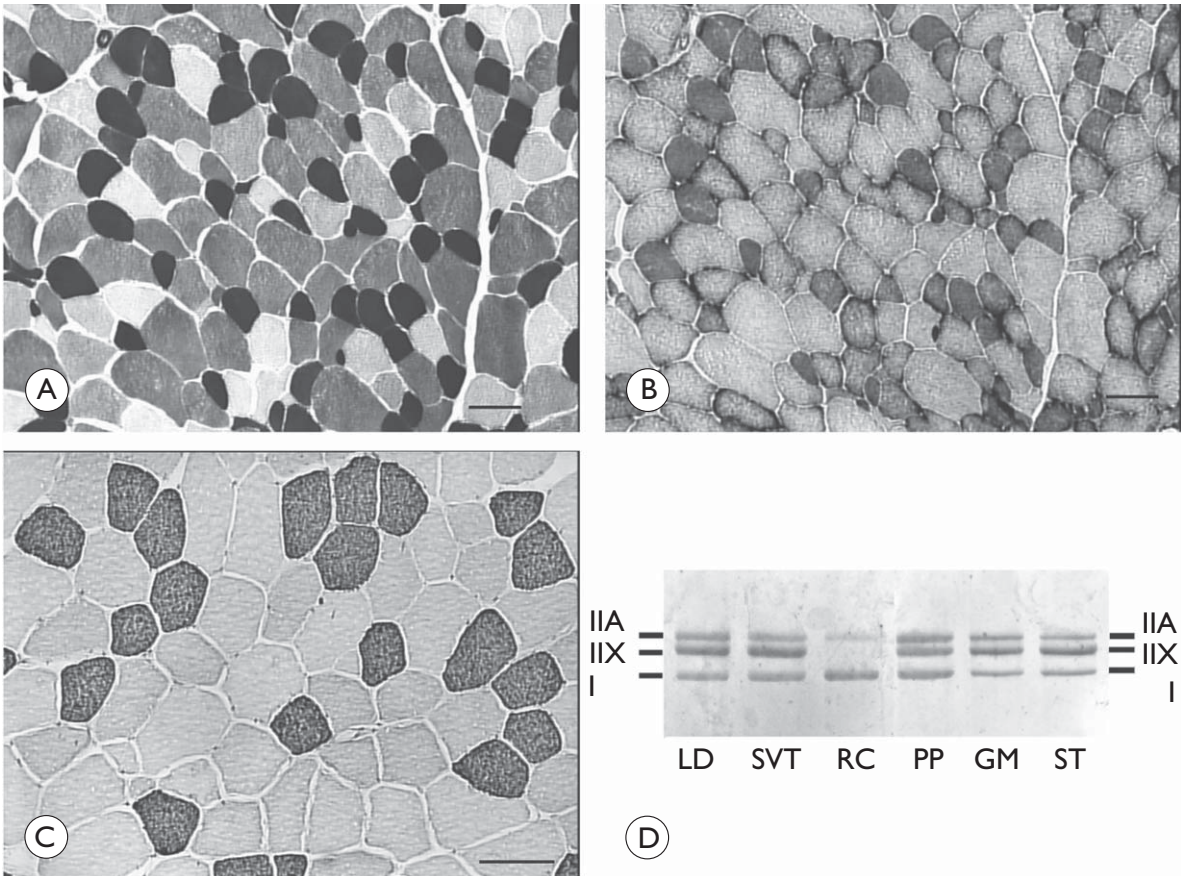
**Fig. 2.1.3**

Illustration of three integrated approaches for investigating skeletal muscle samples in horses: histochemistry (A, B), immunohistochemistry (C), and gel electrophoresis (D). (A) Transverse section of a gluteus medius muscle biopsy stained for demonstration of adenosine triphosphatase activity after acid pre-incubation (pH 4.4); the differential staining allows identification of the various fiber types. (B) Serial section of the previous sample stained to demonstrate succinate dehydrogenase activity; this histochemical stain is performed to examine the oxidative capacity of myofibers. (C) Transverse section of the same sample stained by immunohistochemistry with a monoclonal antibody to the β -slow (type I) myosin heavy chain isoform; this method enables more objective delineation of muscle fiber types than histochemistry. (D) Coomassie blue staining to show myosin heavy chain composition of whole-muscle extracts on 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis; isoforms are identified as IIA, IIX, and I going from the slowest (highest) to the fastest band. LD, M. latissimus dorsi; SVT, M. serratus ventralis thoracis; RC, M. rhomboideus cervicis; PP, M. pectoralis profundus; GM, M. gluteus medius; ST, M. semitendinosus. Scales in A–C, 50 μ m.

Cellular and molecular diversity within equine muscle has also been addressed via study of myofibrillar and non-contractile proteins by immunohistochemistry,^{25,26} gel electrophoresis,²⁷ a combination of both techniques,^{28–30} and enzyme-linked immunosorbent assay.^{31,32} The past few years have seen the production of a considerable number of monoclonal antibodies that label contractile and non-contractile muscle isoproteins, some of which can be used effectively in immunohistochemical evaluation of horse muscle.^{33–35} The technique's specificity provides, among other things, a more objective way to assess muscle fiber type (Fig. 2.1.3C).³⁶ Electrophoretic methods for the quantification of myosin isoforms have also been validated in the horse (Fig. 2.1.3D),³⁷ and immunoelectrophoresis has enabled the specific identification and relative quantitation of certain muscle-derived proteins.³⁸

Molecular biology and genomic techniques have also recently been applied to the field of equine muscle physiology.^{39–41} Northern blotting, reverse transcription followed by polymerase chain reaction, in situ hybridization, and direct sequencing of muscle-specific genes provide the means with which to study the molecular and genetic diversity of muscle proteins at the transcript (mRNA) and cDNA levels, and microarray technology will enable a more global approach. Since during the early phase of transformation between fiber types, isoform-specific mRNA should be detectable some time before a change in the specific protein,⁴¹ this proves invaluable for examining exercise and training effects.^{41,42}

Other techniques

In addition to cellular and molecular techniques, non-invasive analysis, such as nuclear magnetic resonance and electromyography,^{43,44} is increasingly utilized to examine the effects of exercise and training on the neuromuscular system. Furthermore, electromyography, force plate, and gait analysis have applications for assessing muscle activation patterns during locomotion.^{10,45}

Muscle structure and function

Morphology

Development

Most skeletal muscles are derived from paraxial mesodermal tissue following its condensation into

segmentally arranged somites. Cells of certain lineages become compartmentalized within each somite as it differentiates; the dorsolateral compartment, known as the myotome, contains two subsets of myogenic precursor cells. The cells of one subset are destined to become the axial musculature, whereas cells of the other subset migrate into the periphery to form the muscles of the body wall and the limbs.⁴⁶ Myogenic precursor cells differentiate to form myoblasts, and these fuse to become discrete populations of myotubes that subsequently fuse into myofibers,^{47,48} while α -motor neurons establish neuromuscular junctions. Embryonic myogenesis shares many similarities with the regeneration of mature myofibers following injury, a subject that is covered in detail elsewhere.⁴⁷

Gross anatomy

Locomotor muscles are mainly located proximally on the appendicular skeleton, which has the effect of reducing the weight of the lower limb and decreasing the energy necessary to overcome inertia when the limb swings back and forth.^{49,50} Movements of the distal limb are mainly passive and result from the release of elastic energy of the digital flexor tendons and suspensory ligament when the limb is unloaded.⁵¹ However, myofibroblasts (with contractile properties) have been observed in these tendons.⁵² Wilson & Watson⁵³ have shown that thoracic limb protraction is largely a passive process in the horse. They liken thoracic limb protraction to a catapult mechanism in which energy is stored relatively slowly in elastic tissues (the internal tendon of biceps brachii and lacertus fibrosus) during limb loading, but released rapidly when the toe leaves the ground, thereby quickly protracting the limb. In contrast, movements of the proximal limb result from active muscular contraction.⁵⁴ In general, most locomotor muscles are active during the propulsion stage of weight bearing in each limb.⁵⁵ Muscle activity, as measured by electromyography, is positively correlated with running speed, but shows that muscles within the same group have significant differences in their activities when a horse exercises at constant speed.⁵⁶

More than 90% of a muscle is made up of myofibers, with the rest consisting of nerves, blood vessels, and the fat and connective tissue that separate the individual fibers (endomysium), the fascicles (perimysium), and the whole muscle (epimysium; see Fig. 2.1.1). The connective tissue merges with both the origin and the insertion tendons of the muscle, as well as with internal tendons in compartmentalized muscles. Blood vessels and nerves course within the

perimysium. Capillary arrangement in the skeletal muscle is optimized for oxygen delivery to tissue during exercise;⁵⁷ usually several capillaries are located within close proximity, sometimes circumferentially but more often running parallel to each fiber. The internal muscular architecture varies considerably both within and between equine muscles.^{9,58,59} For example, different fiber lengths and pennation angles significantly affect power output and the velocity of shortening of specific muscles, in accordance with their specific function.^{9,60} Regional functional architecture and specialization of muscles of the pelvic and thoracic limbs have also been reported in horses.^{9,61–63} In general, the proximal limb is characterized by muscles with large volumes, long and parallel fascicles, and extremely short tendons/aponeuroses, whereas the distal limb is characterized by muscles with small volumes, short, pinnate fascicles, and long tendons. Hence, in general, proximal limb musculature is specialized for work output, while the distal limb musculature is specialized for generating force economically.

Histology

The skeletal myofiber is an elongated cell (generally believed to be between 30 and 100 mm in length) with tapered ends. Fibers vary from 10 to 100 μm in diameter and are multinucleated (Fig. 2.1.4A). The nuclei are normally located at the fiber's periphery in a subsarcolemmal position. Although the cytoplasm contains other organelles found in many cell types, it is largely occupied by the contractile apparatus, which consists of contractile proteins and their supportive structures all grouped together as myofibrils. In longitudinal sections, individual muscle fibers have numerous cross-striations (dark and light bands), orientated perpendicular to the fiber's long axis (Fig. 2.1.4B). Lighter I bands alternate with darker A bands; within the I band there are dense striations called Z disks.

Ultrastructure

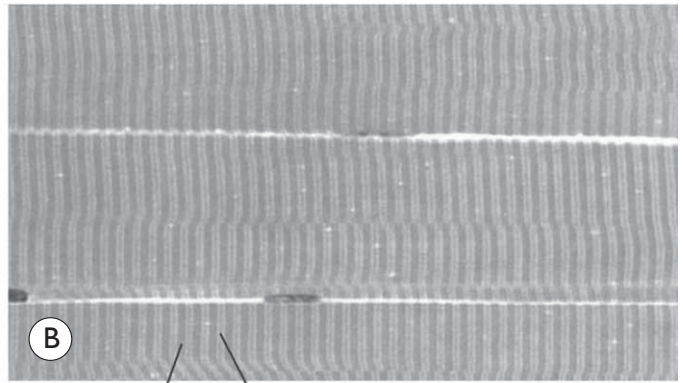
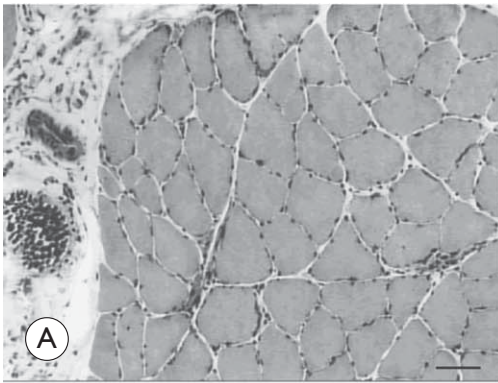
The repeating unit between two adjacent Z disks is known as a sarcomere, the unit of muscular contraction. Each sarcomere includes half the I band on each side of the A band (Fig. 2.1.4C,D). I bands contain only thin filaments (8 nm diameter), whereas the A bands contain both thin and thick (15 nm diameter) filaments. Within the A band, the H band is defined as the central area where the thick filaments do not overlap with thin filaments (Fig. 2.1.4D). The central darker portion of the H band is designated as the M line (see Fig. 2.1.4D). Transverse section of the sarcomere at

the overlapping zone between thick and thin filaments reveals each thick myofilament surrounded by thin myofilaments arranged in a hexagonal lattice (Fig. 2.1.4E). Muscle contraction occurs when, within each sarcomere, thin myofilaments slide over the thick myofilaments, bringing the adjacent Z disks closer together. Hence, upon contraction, the I band shortens and the H band starts to disappear.

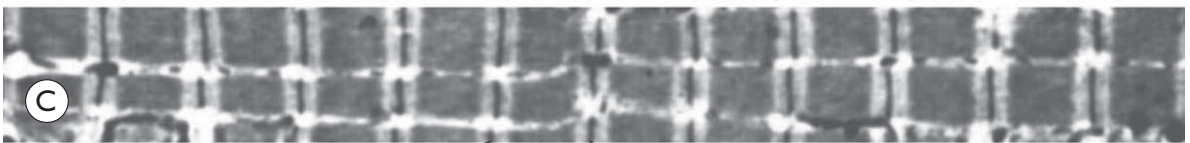
Thick filaments contain myosin and other myosin-binding proteins. Sarcomeric myosins have two heads and a long tail and consist of two heavy chains and two pairs of light chains (Fig. 2.1.5A). The myosin head is the motor domain that contains the ATP-binding site, the actin-binding site and the myofibrillar ATPase enzyme. The major components of the thin filaments are tropomyosin, the troponin complex (consisting of three subunits: troponin C (TnC), troponin T (TnT) and troponin I (TnI)), and two helical filamentous strands of actin (F-actin), made up of polymerized globular actin monomers (G-actin) (Fig. 2.1.5B). Elongated tropomyosin dimers lie within the major groove of the actin filament, spanning seven actin monomers; each troponin complex is also associated with a seven-actin repeat. The elongated NH_2 -terminal of TnT extends for a considerable length from each tropomyosin molecule, spanning the gap between adjacent molecules.

Mitochondria are located beneath the sarcolemma and between myofibrils (Figs 2.1.6, 2.1.7). This intimate relationship means that ATP produced during oxidative phosphorylation is readily available for the contractile machinery. Intramuscular substrates, such as glycogen and lipids, are also stored between the myofilaments and under the sarcolemma (see Fig. 2.1.7). Numerous other proteins, including myoglobin, glycolytic enzymes, and various intermediate filaments, are distributed throughout the cytoplasm.

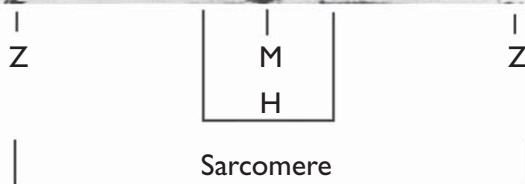
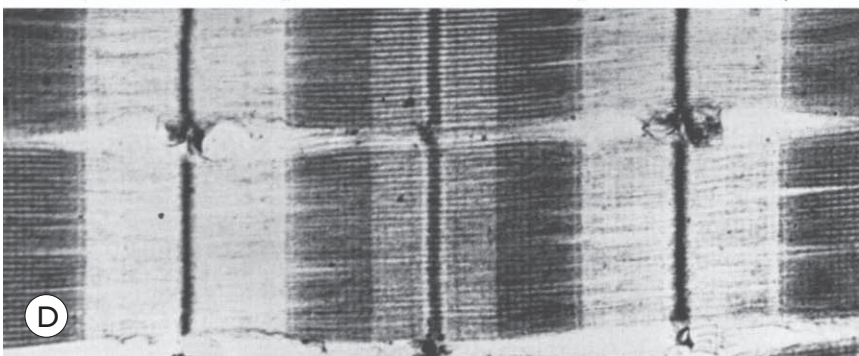
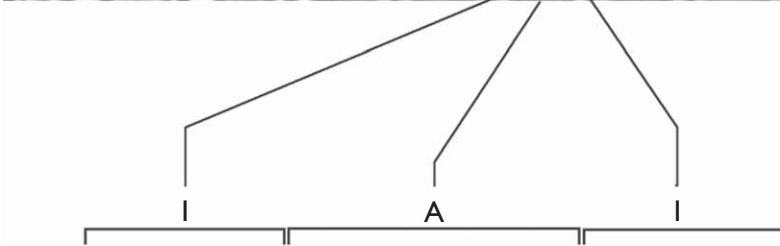
The sarco(endo)plasmic reticulum (SR) of skeletal myofibers is an intracellular membranous system located between the myofibrils (see Figs 2.1.6, 2.1.7), but which has no physical continuity with the external surface membrane (sarcolemma). Much of its tubular network is orientated longitudinally to the myofibrils. The SR contains, amongst other molecules, a large amount of the enzyme Ca^{2+} -ATPase, the protein calsequestrin, and the calcium release channel (ryanodine receptor or RYR1). At the AI junction of the sarcomere, the SR tubules become confluent and form terminal cisternae orientated perpendicularly to the long axis of the cell (Fig. 2.1.7D). Two adjacent cisternae are separated by a structure known as the T-tubule, which is a long tubular invagination of the



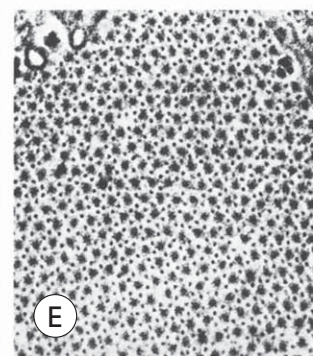
Myofibers



Myofibrils



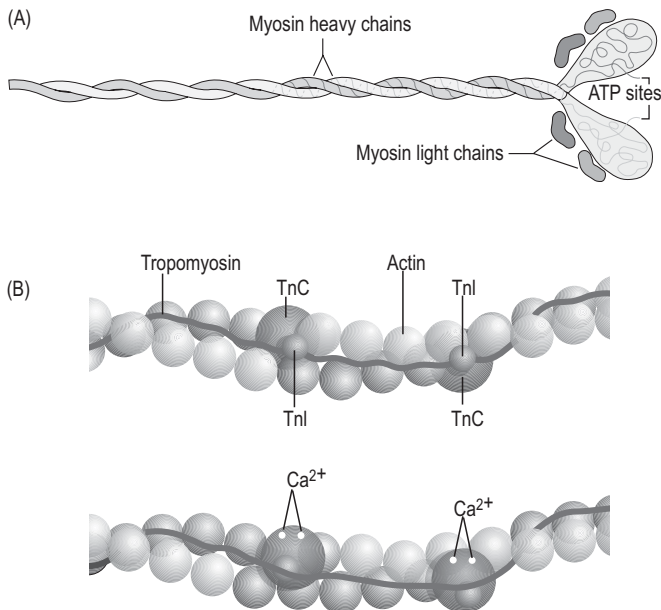
Sarcomere



Myofilaments

Fig. 2.1.4

Organization of the contractile apparatus from the cellular to the molecular level. (A) Transverse section from a specimen of the *M. sacrocaudalis dorsalis medialis* stained by hematoxylin and eosin and examined by light microscopy; scale = 50 μm . (B) Longitudinal section from the same specimen with the same stain. (C) Striated aspect of myofibrils when observed by electron microscopy at very low magnification, showing Z disks, A bands, and I bands of sarcomeres. (D) Electron micrograph of longitudinal sectioned myofibrils at $\times 30\,000$ magnification. (E) Electron micrograph of transverse sections of one myofibril in the overlap zone (A band) between thick and thin myofilaments of sarcomere, illustrating the hexagonal arrangement of these filaments, $\times 150\,000$.

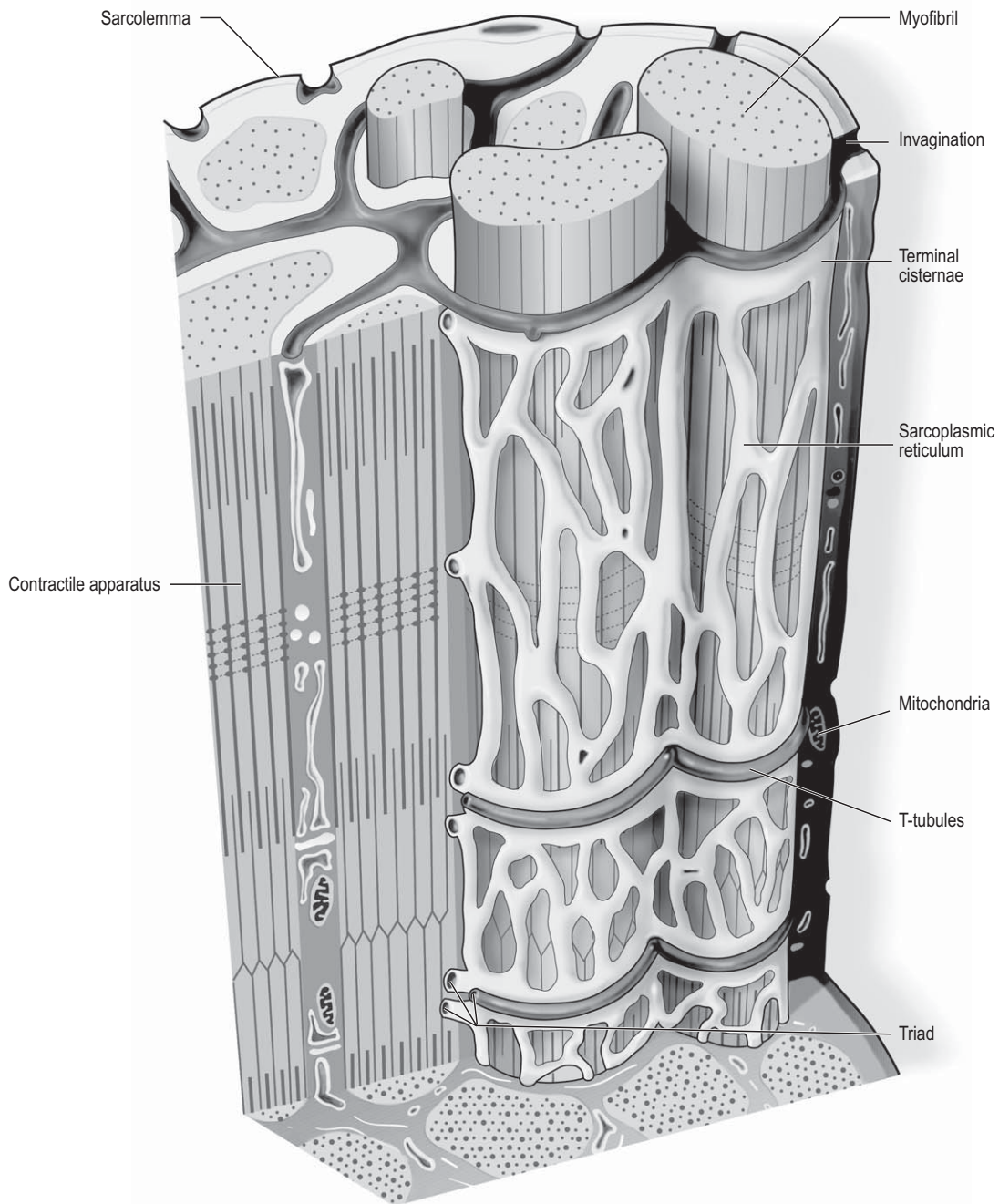
**Fig. 2.1.5**

(A) Diagram of the myosin molecule illustrating the α -helical coiled-coil tail, the folding of each heavy chain to form a globular head, the site of ATP hydrolysis, and the location of the four myosin light chains (regulatory and essential). (B) Model of the troponin–tropomyosin–actin that make up the thin myofilaments, during muscle relaxation (above) and contraction (below). In the relaxed state, the inhibitory region of troponin (TnI) is attached to actin and tropomyosin, whereas troponin C (TnC) is bound to Mg^{2+} . In this conformation, myosin cannot bind. In the contracted state, two Ca^{2+} ions bind to TnC, which in turn interacts with TnI. A conformational change to the troponin–tropomyosin complex exposes the myosin-binding sites, thereby allowing the power stroke to occur.

sarcolemma communicating directly with the extracellular space. Together the three structures make up a triad (see Figs 2.1.6, 2.1.7).

The motor end-plate (see Fig. 2.1.11B) is a specialized region on each fiber where the α -motor neuron interdigitates with the sarcolemma. The postsynaptic membrane of the motor end-plate contains numerous acetylcholine receptors. The remainder of the

sarcolemma contains a variety of specific structural proteins, channels, pumps, and hormone receptors. Of note, myofibers have a cytoskeleton of various intermediate filament proteins that link the contractile apparatus with a complex of proteins at the sarcolemma, known as the dystrophin-associated complex (see Fig. 2.1.14), which itself provides a structural link with the extracellular matrix. Between the

**Fig. 2.1.6**

Diagrammatic representation of the internal structure of a muscle fiber. Myofibrils are surrounded by the net-like tubular sarcoplasmic reticulum (SR); the SR converges at the junction between A and I bands of sarcomeres to form terminal cisternae. Between each pair of terminal cisternae there is an invagination of the sarcolemma called the T-tubule. Together, the three structures make up a triad. Mitochondria are dispersed between myofibrils.

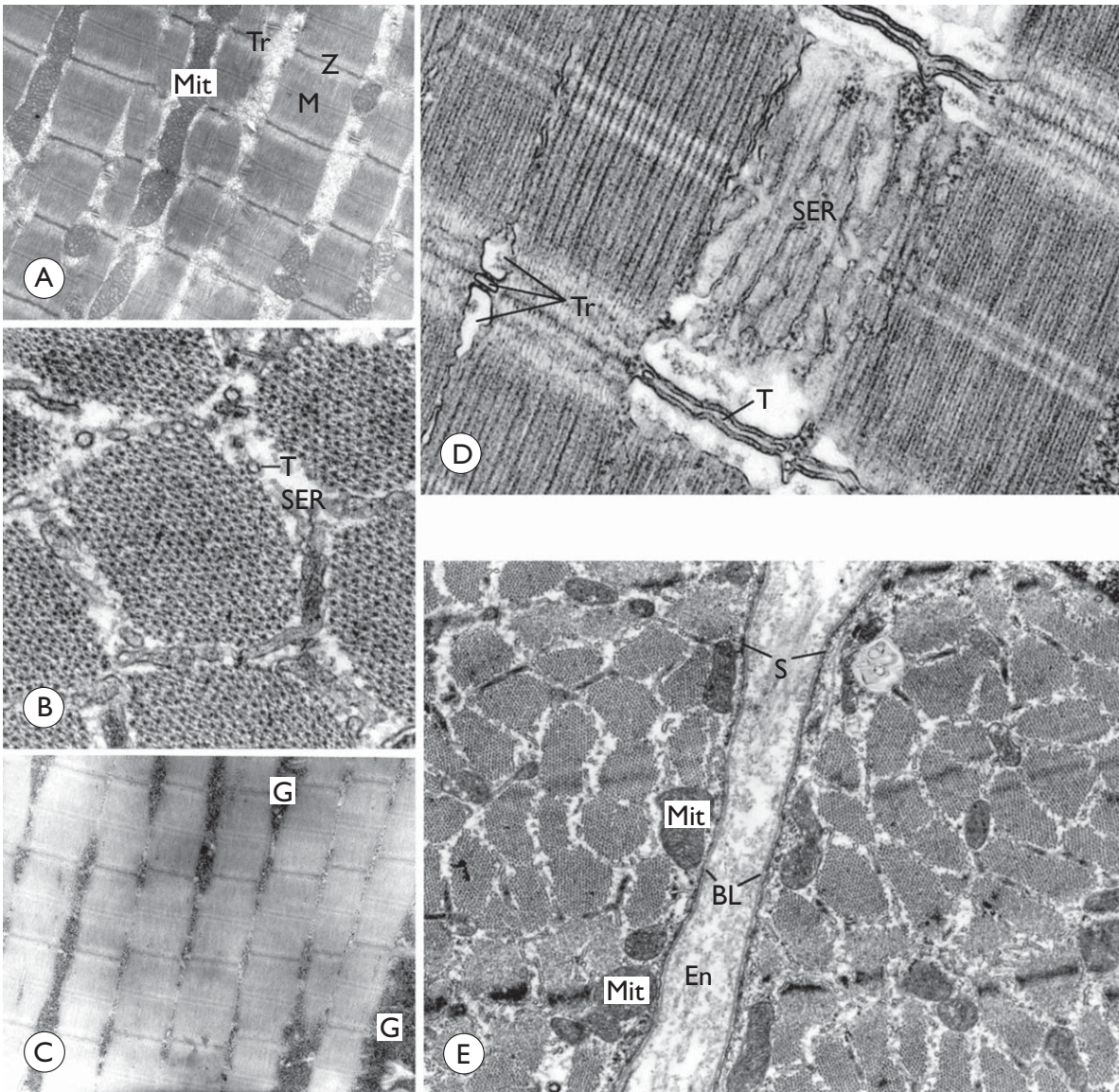


Fig. 2.1.7

Ultrastructural appearance of equine skeletal muscle cells when observed by electron microscopy. (A) Type IIA myofiber sectioned longitudinally; note the straight and thin appearance of Z lines, M lines in the middle of each sarcomere, a triad (Tr), and abundant mitochondria (Mit) within the intermyofibrillar spaces. (B) Transverse sectioned myofibrils showing the arrangement of the sarco(endo)plasmic reticulum (SER) and T-tubules (T); magnification $\times 54\,000$. (C) Type IIX muscle (as determined by immunoelectron microscopy — not shown) fiber sectioned longitudinally; note the abundant glycogen granules (G); magnification $\times 25\,312$. (D) Electron micrograph of an equine sarcomere showing the arrangement of the sarco(endo)plasmic reticulum (SER), T-tubule (T), and a triad (Tr); magnification $\times 58\,125$. (E) Two adjacent equine myofibers sectioned transversely showing the sarcolemma (S) and the basal lamina (BL), interposed between the sarcolemma and the extracellular matrix of the endomysium (En); there are also abundant mitochondria (Mit) located beneath the sarcolemma and between myofibrils; magnification $\times 35\,835$. (Frames A and C are courtesy of Drs LH Sucre and HJ Finol from the Universidad Central de Venezuela. Frames B, D, and E are courtesy of Dr A Blanco from the University of Cordoba.)

sarcolemma and the extracellular matrix is the basal lamina (Fig. 2.1.7E), which is generally closely apposed to the sarcolemma except where it leaves the sarcolemma to course over the surface of satellite cells (Fig. 2.1.8). Further information about satellite cells is available elsewhere.⁴⁷

General muscle physiology

The motor unit

A motor unit consists of an α -motor neuron and the skeletal muscle fibers that it innervates (Fig. 2.1.9).⁶⁴ Since each time an α -motor neuron fires, the entire motor unit contracts, coarser movements are generated in those muscles that have many muscle fibers making up motor units (such as the locomotor muscles) compared with those with few (such as the extrinsic eye muscles). Muscle fibers within one motor unit are generally all of the same histochemical type, but they are normally widely distributed between fibers from other motor units and therefore give rise to the characteristic checkerboard pattern that is apparent with certain histochemical stains (see Fig. 2.1.3A). Loss of this checkerboard pattern is evident in denervated muscle (Fig. 2.1.10A,B) where there may be selective loss and atrophy of fibers of one histochemical type (Fig. 2.1.10A), or in reinnervated muscle following disease or injury, by patterns of fiber grouping (Fig. 2.1.10C).⁶⁵ Large-diameter α -motor neurons innervate fast-twitch fibers, whereas smaller ones tend to innervate slow-twitch fibers.

Contractile force for a particular muscle is partly regulated by the rapidity of neuron discharge; muscle fibers contract with a twitch following a single discharge of a motor neuron, but sustained contraction (tetanus) results from repetitive neuron firing. The force of a contraction increases with the rate of discharge up to a maximum limit that is determined by the properties of the muscle. Furthermore, a process known as recruitment, which reflects the gradual inclusion of larger motor neurons as greater force is required, also regulates force (see below). Relatively weak and slow contractions required for maintenance of posture therefore involve small-diameter α -motor neurons and slow-twitch fiber types, whereas locomotion and rapid movements rely on recruitment of larger diameter α -motor neurons and fast-twitch fibers.

Muscle proprioception

Proprioception is the term given to the mechanism underlying the self-regulation of posture and move-

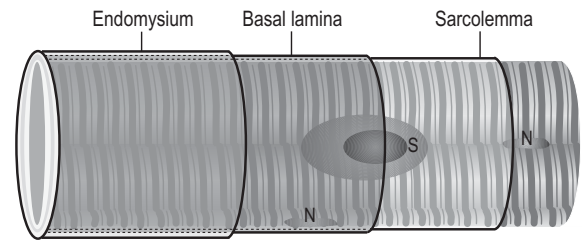


Fig. 2.1.8

Diagram illustrating the relationship of a satellite cell (S) to the sarcolemma, basal lamina, and endomysium. N, myonucleus.

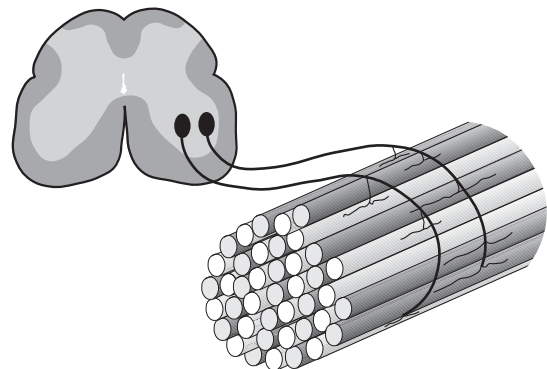


Fig. 2.1.9

Diagram illustrating the organization of motor units. A motor neuron from the ventral horn of the spinal cord supplies the motor innervation to a group of muscle fibers with similar contractile and metabolic properties.

ment through stimuli originating in sensory receptors embedded in joints, tendons, muscles, and the labyrinth of the ear. In skeletal muscles and tendons these receptors are known as spindles and Golgi tendon organs and each type has been well characterized in the horse.^{66–69} Signals derived within these sensory receptors are conveyed via a variety of well-defined reflex pathways that generate specific motor responses.

Muscle spindles consist of specialized intrafusal muscle fibers surrounded by a connective tissue capsule and lie parallel to regular muscle fibers (Fig. 2.1.11A). Sensory nerves (type Ia and type II) terminate on the intrafusal fibers in specialized sensory endings and generate afferent signals that are relayed to the spinal cord via the dorsal horn. Type Ia nerves carry both dynamic (rate of stretch) and static (amount of stretch) afferent signals, whereas type II nerves

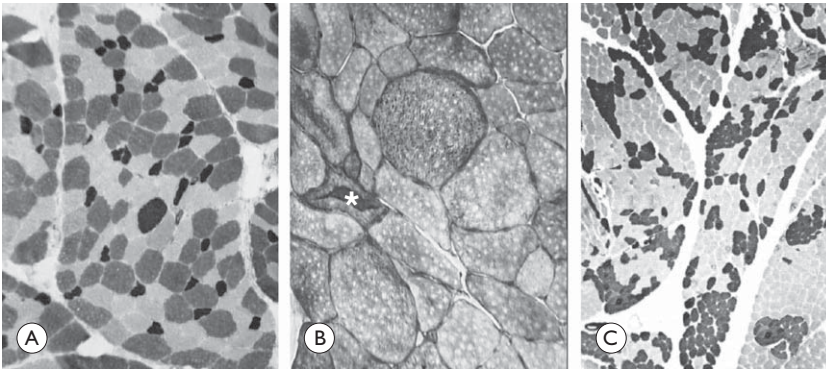


Fig. 2.1.10

Histopathological evidence of neurogenic muscle atrophy in the horse. (A) Transverse section of the M. gluteus medius (7 cm depth) stained with ATPase at pH 4.5 from one horse with motor neuron disease; note the general, but particularly type I (black) fiber, atrophy. (B) Transverse section of the M. vastus lateralis stained with periodic acid–Schiff (PAS) from a horse with femoral nerve paralysis; the asterisk shows a fiber with a target structure in its center, suggesting reinnervation following denervation. (C) Transverse section of the M. cricoarytenoideus dorsalis stained with ATPase (pH 9.4) from a horse with laryngeal hemiplegia showing disruption of the normal checkerboard pattern and fiber type grouping.

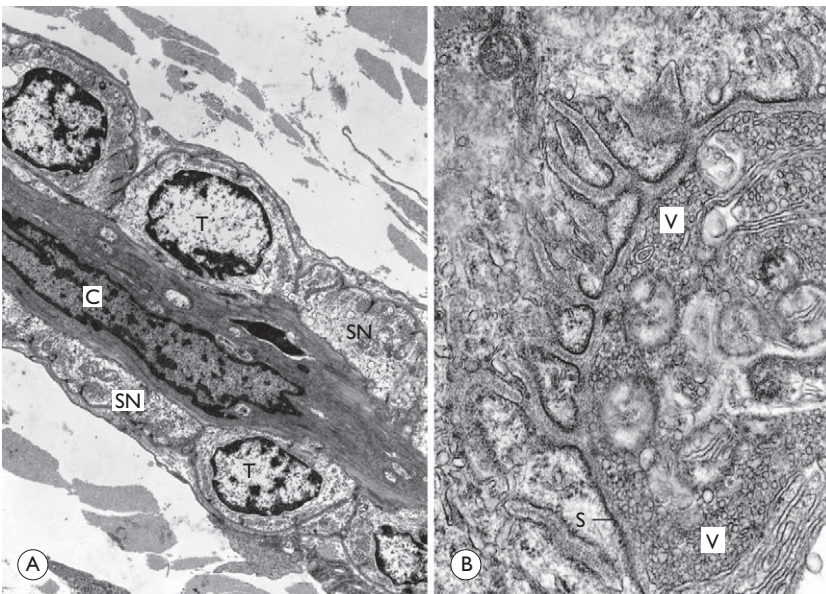


Fig. 2.1.11

(A) Electron micrograph of an equine muscle spindle; sensory nerves (SN) appose the abundant teloglia cells (T) which are surrounded by an internal capillary (C); magnification $\times 37\,125$. (B) Electron micrograph of an equine myofiber showing the neuromuscular junction (end-plate) of the sarcolemma (S); note the clear (acetylcholine-containing) vesicles (V) in the pre-synaptic nerve terminal; magnification $\times 43\,000$. (Courtesy of Dr A Blanco from the University of Cordoba.)

sense only static muscle length. Motor innervation to the muscle spindle is provided by γ -motor neurons, which regulate the sensitivity of muscle spindles to muscle stretch. Golgi tendon organs are located within the connective tissues of tendons, joint capsules, and muscles. They lie in parallel with the direction of mechanical force that they measure and, from them, afferent type Ib fibers convey proprioceptive signals to the spinal cord.

Electrical and ionic properties of the sarcolemma

The sarcolemma maintains the interior of the fiber at a negative potential (the membrane potential) when compared to the extracellular fluid while the fiber is in a resting state. The negative potential is derived from the disequilibrium of ionic concentrations (mostly Na^+ and K^+) across the membrane and is generated partly by the action of the Na^+/K^+ ATPase pump, which extrudes three Na^+ ions for every two K^+ ions taken up. This results in the cytoplasm having a much higher K^+ concentration but much lower Na^+ concentration than the extracellular fluid. The remainder of the membrane potential is derived from the tendency of ions to diffuse down their electrochemical gradients across the semipermeable membrane.

Acetylcholine released from pre-synaptic nerve terminals at neuromuscular junctions (end-plates; Fig. 2.1.11B) binds to acetylcholine receptors and increases the conductance of the post-junctional membrane to Na^+ and K^+ . The inward movement of Na^+ down its concentration gradient predominates, causing a transient depolarization (about 20 mV) in the end-plate, known as the end-plate potential. This depolarization is sufficient to activate sarcolemmal voltage-gated Na^+ channels — the channels that are mutated in hyperkalemic periodic paralysis (reviewed elsewhere⁴⁷). These Na^+ channels open eliciting propagation of an action potential along the membrane. Following this, and in response to depolarization, voltage-gated K^+ channels open, resulting in the downswing of the action potential. The action potential therefore conducts rapidly along the sarcolemma in a wavelike fashion away from the neuromuscular junction.

Excitation–contraction coupling

Action potentials are conducted into the interior of muscle fibers via the T-tubules and there activate voltage-gated channels known as dihydropyridine receptors (DHPR). Unlike in cardiac muscle, very little calcium enters the muscle fiber from the extracellular space (via the DHPR).⁷⁰ Instead, a mechanical link

between DHPR and the SR Ca^{2+} release channel (ryanodine receptor, RYR1) at the junctional feet of the triads causes release of calsequestrin-bound Ca^{2+} from the SR's interior (Fig. 2.1.12). A positive feedback loop, known as calcium-induced calcium release, is responsible for further activation of RYR1, with the result that the calcium concentration within the cytoplasm increases about 100-fold from a resting concentration of approximately 50 nM.⁷⁰ The anatomical location of the terminal cisternae results in Ca^{2+} being released adjacent to the overlapping contractile apparatus.

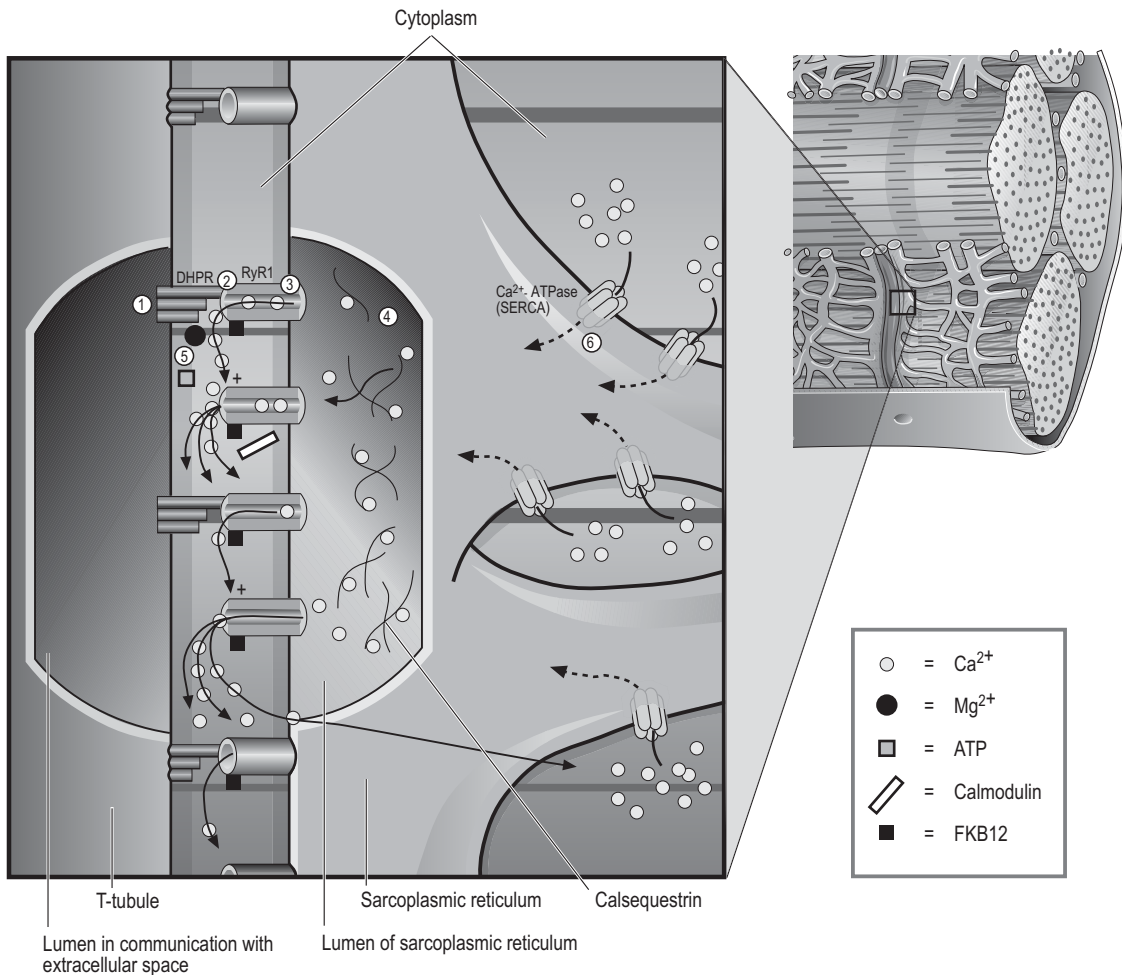
Binding of Ca^{2+} to high-affinity binding sites on troponin C causes a conformational change to the troponin–tropomyosin complex (see Fig. 2.1.5B). This results in the exposure of the myosin-binding sites on F-actin and allows the myosin globular head to attach as ATP is hydrolyzed, thus forming the crossbridge. Force generation and shortening of the sarcomere occur as a result of a conformational change of the myosin head. Adenosine diphosphate and inorganic phosphate are displaced by actin, which is followed by dissociation of the actin–myosin bridge due to the binding of ATP to myosin again. The crossbridge cycle continues while the cytoplasmic Ca^{2+} concentration remains high. Relaxation is achieved when the Ca^{2+} is resequenced within the SR via the action of the Ca^{2+} -ATPase pumps (see Fig. 2.1.13 for a summary).

Force transmission

The force that is generated in the crossbridge cycle is transmitted via the contractile apparatus to intermediate filament proteins that act to maintain and stabilize the muscle fiber's shape during contraction. These intermediate filaments provide a structural link first to the sarcolemma and then to the extracellular matrix via a group of proteins known as the dystrophin-associated protein complex (Figs 2.1.14, 2.1.15).⁷¹ Contractile forces are transmitted from each muscle fiber via the extracellular matrix and the connective tissues of tendons, and ultimately to the bones of the skeleton.

Oxygen availability

ATP replenishment in (predominantly) oxidative fibers requires a readily available source of oxygen that is provided by the O_2 -binding heme protein known as myoglobin.^{72,73} The P_{50} for equine myoglobin (the P_{O_2} when it is 50% saturated) is about 2.4 mmHg at physiological temperatures and pH,⁷⁴ and therefore far to the left of hemoglobin and close to the P_{O_2} of muscle cells. During exercise, oxygen demand rises dramatically, met by a 20–30 times increased blood

**Fig. 2.1.12**

Depolarization of the T-tubule membrane during the action potential activates voltage-gated Ca^{2+} channels (1) (dihydropyridine receptors, DHPR) in the wall of the T-tubule. A mechanical link (2) with ryanodine receptors (RyR1) (3) located in the wall of the sarcoplasmic reticulum causes them to open and release calsequestrin-bound Ca^{2+} ions from the lumen of the SR (4). This Ca^{2+} stimulates further Ca^{2+} release via the calcium-induced calcium release mechanism. The process is modulated by other factors within the cytoplasm that include ATP, calmodulin, and Mg^{2+} (5). After release into the cytoplasm, calcium activates the contractile apparatus by binding to troponin-C (Fig. 2.1.13). Reuptake into the SR occurs via the Ca^{2+} -ATPase pumps (6).

flow through the muscle capillary beds.³ Capillary dilation results partly from autonomic control and stretch imposed by the higher blood pressure, but also follows the local production of vasoactive substances that include potassium, adenosine, and nitric oxide. The latter is produced by nitric oxide synthase, found both in the endothelium of the capillaries and bound to the dystrophin-associated protein complex within the contracting muscle fibers themselves (Fig. 2.1.16).⁷⁵

Energy provision for muscular functions

Muscles cannot contract without a biochemical source of energy provided by the cleavage of high-energy phosphate bonds within ATP. In addition to the normal cellular metabolic requirements and the energy needed for ion pumping up concentration gradients, ATP is required in the contractile cross-bridge cycle; at the head of each myosin molecule, an ATP molecule is hydrolyzed, releasing energy (E)

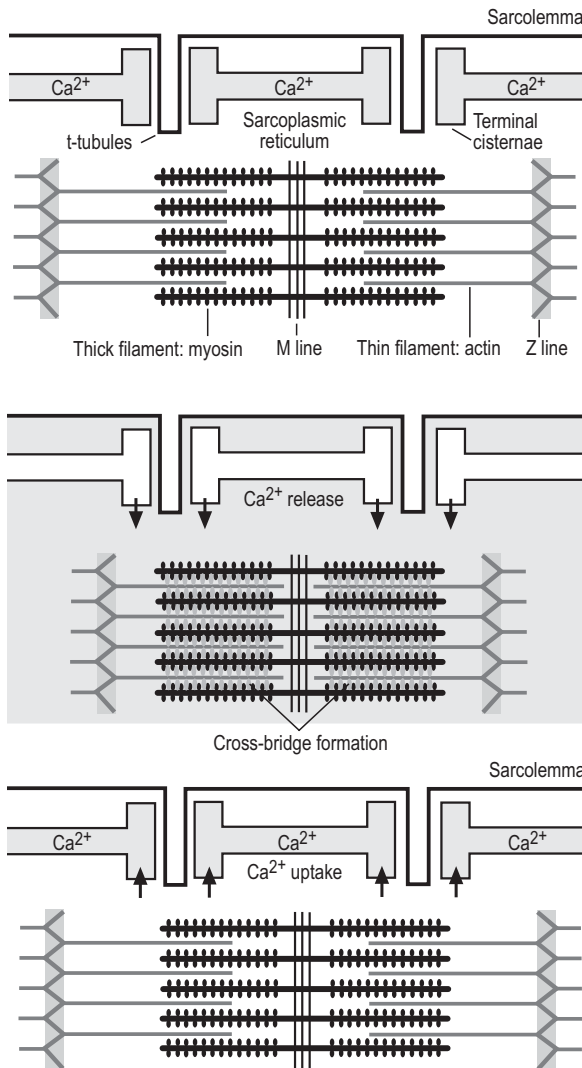


Fig. 2.1.13

Diagram illustrating excitation–contraction coupling and the role of calcium in the contractile mechanism. (Top) Calcium stored in the SR during the relaxed state of the muscle. (Middle) A wave of depolarization moves along the sarcolemma and alters the membrane potential of the T-tubules. The altered membrane potential culminates in movement of Ca^{2+} ions into the cytoplasm from the SR (see Fig. 2.1.12). Thin filament inhibition is removed, allowing the globular heads of myosin to interact with actin and form a crossbridge that results in shortening of the total length of sarcomere. (Bottom) Muscle relaxation is achieved when Ca^{2+} is pumped back into the SR.

in a reaction catalyzed by the enzyme actomyosin ATPase:



where ADP is adenosine diphosphate and Pi is inorganic phosphate.

Aerobic pathways

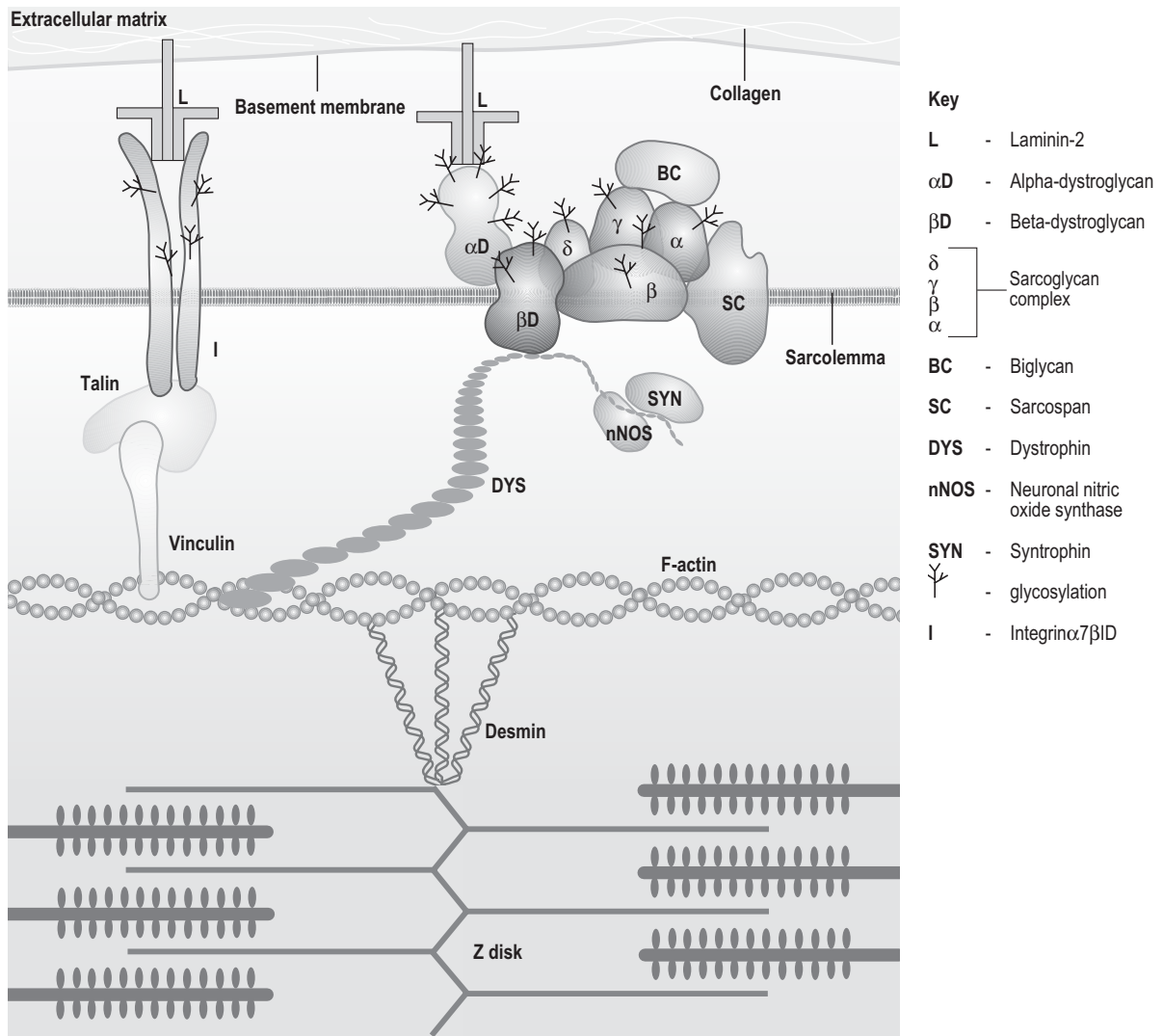
Within mitochondria, β -oxidation of free fatty acids (FFA), the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation (via the electron transport chain) combine to produce ATP aerobically (Fig. 2.1.17). During the process, the coenzymes nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) are reduced to NADH_2 and FADH_2 , respectively. Subsequently, NADH_2 and FADH_2 are reoxidized to NAD and FAD via the electron-transport chain in which oxygen acts as the final hydrogen acceptor to form water. Oxygen dissolved within the cytoplasm and bound to myoglobin is rapidly used up and must be replenished; hence oxidative phosphorylation depends on a dense capillary network between muscle fibers (Fig. 2.1.18).

Acetyl-CoA is the substrate for the TCA cycle and its complete oxidation results in the formation of 12 ATP molecules. Acetyl-CoA is derived from pyruvate following anaerobic metabolism of glucose and glycogen within the cytoplasm (glycolysis) (see Fig. 2.1.17); at submaximal exercise intensities, most pyruvate produced via glycolysis is transported into mitochondria and is converted to acetyl-CoA, which enters the TCA cycle. Thirty-six molecules of ATP are produced in the complete breakdown of glucose via these pathways. However, acetyl-CoA is also derived from the oxidation of fatty acids, following their mobilization from the liver or adipose tissue. Beta-oxidation of FFA is highly efficient, as complete oxidation provides up to 146 molecules of ATP.

Anaerobic pathways

In addition to the pathways described above, additional anaerobic mechanisms exist for ATP replenishment in muscle. They can be divided into two different mechanisms. The first system involves high-energy phosphate transfer in the coupling of the creatine kinase (1), adenylate kinase (2) and AMP deaminase (3) enzyme systems:

1. $\text{ADP} + \text{phosphocreatine} \rightarrow \text{ATP} + \text{creatine}$
2. $\text{ADP} + \text{ADP} \rightarrow \text{ATP} + \text{AMP}$
3. $\text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_3$.

**Fig. 2.1.14**

Interconnections between the contractile apparatus, the dystrophin-associated complex (DAC), and the extracellular matrix. Further structural support is provided by integrin molecules that span the sarcolemma. Note nNOS makes up part of the DAC. In addition to a structural role, the DAC likely has a role in cell signaling.

The enzymes that catalyze these reactions help buffer ATP concentrations at the expense of lowering the cellular concentration of phosphocreatine and free ADP, while increasing the concentrations of creatine, adenosine monophosphate (AMP) and inosine monophosphate (IMP). These reactions occur in active muscles at top speeds, but provide only a small amount

of ATP for a few seconds. Deamination of adenosine nucleotides leads to the production of ammonia (NH_3), uric acid, and allantoin.⁷⁶

The second anaerobic pathway involves glycolysis acting independently from the oxidative pathways. Glycolysis requires glucose-6-phosphate as a substrate, which may be derived from the

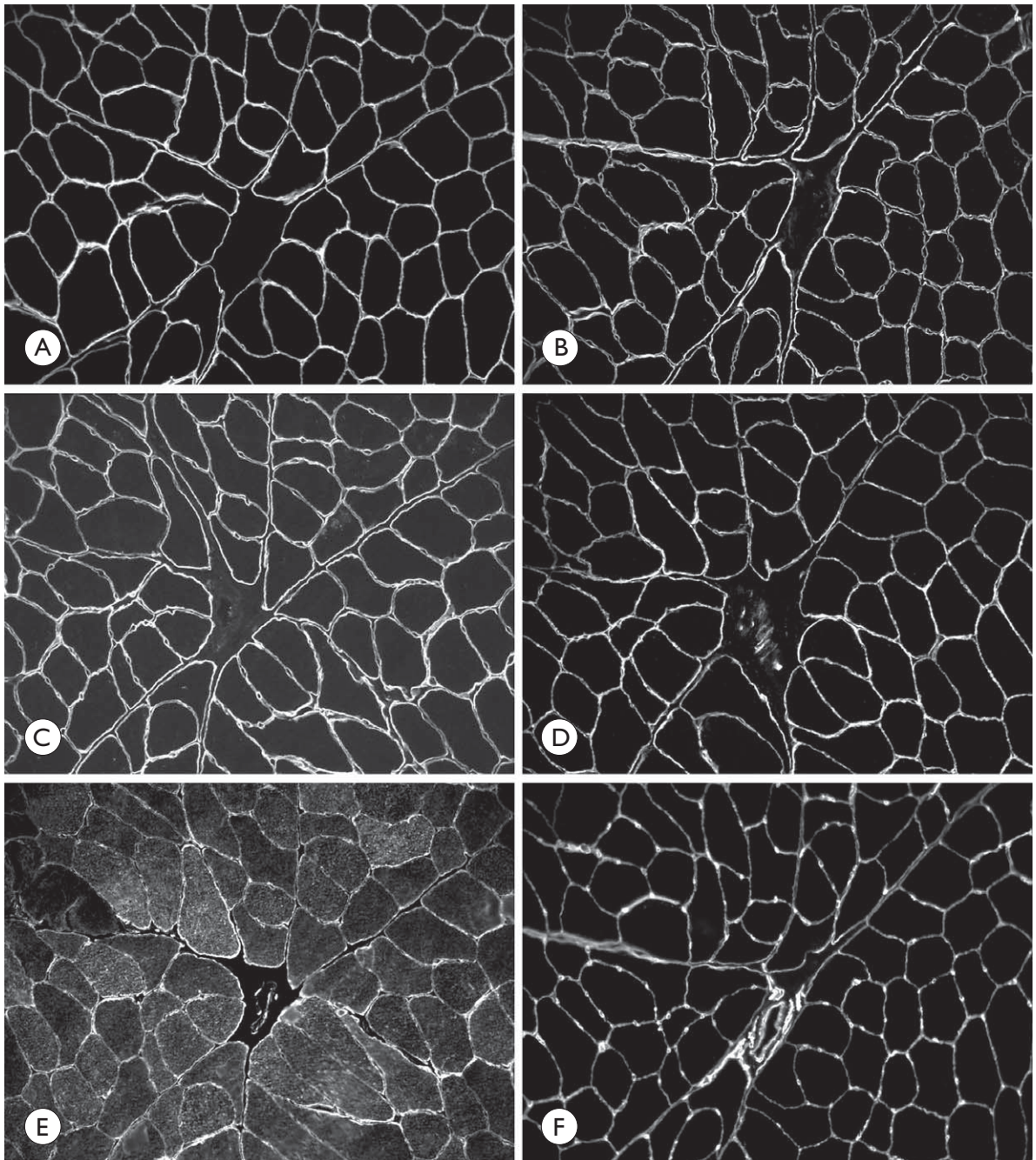


Fig. 2.1.15

Immunofluorescence labeling of serial 7 μm sections from the gluteus medius muscle of a normal Thoroughbred, showing proteins that link the contractile apparatus to the extracellular matrix. (A) Beta dystroglycan; (B) laminin α2; (C) dystrophin; (D) integrin α7β1d; (E) desmin; (F) collagen IV. Working clockwise from desmin (E), contractile force is conveyed via dystrophin (C) to the dystrophin-associated complex (A and B) and ultimately to the extracellular matrix (F). Integrin α7β1d likely also plays a structural role. Compare with Figures 2.1.14 and 2.1.16. Note the capillaries located around each muscle fiber, and a central blood vessel that are also localized with the collagen IV antibody in (F).

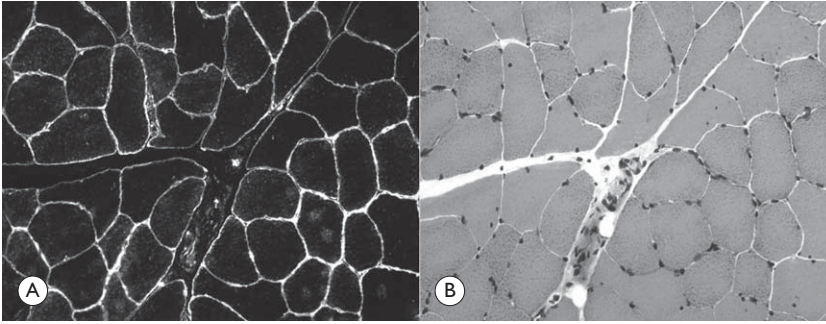


Fig. 2.1.16

Serial 7 μm (A) and 10 μm (B) sections from the gluteus medius muscle of a normal Thoroughbred. (A) Immunofluorescence labeling of neuronal nitric oxide synthase (nNOS). (B) Hematoxylin and eosin stain. Note the sarcolemmal distribution of nNOS, and compare with Figures 2.1.14 and 2.1.15.

phosphorylation of glucose by hexokinase or by the mobilization of stored intracellular glycogen that is metabolized first to glucose-1-phosphate via glycogenolysis (see Fig. 2.1.17) and then converted to glucose-6-phosphate. Blood glucose is transported across the sarcolemma by means of specific glucose transporters that include GLUT-1 and GLUT-4 (see Fig. 2.1.18).⁷⁷ GLUT-1 is normally located within the sarcolemma and provides basal glucose requirements; however, GLUT-4 receptors translocate to the sarcolemma in vesicles, in response to insulin or the demands of exercise (Fig. 2.1.19).⁴⁷ The glycolytic pathways result in the formation of two pyruvate molecules that in the absence of oxygen are converted to lactate.

Integration of aerobic and anaerobic pathways

Aerobic production of ATP is a relatively slow but highly efficient process, while anaerobic pathways produce energy rapidly but relatively inefficiently. Although both pathways are generally active during exercise, the relative contribution within each muscle depends on the nature, intensity level, and duration of the activity, the muscle's fiber-type composition, the availability of oxygen and substrates, and the relative concentrations of intermediary metabolites that may potentially activate or inhibit selected enzymes.^{76,78} Hence, at the beginning of low-speed exercise when oxygen is abundant, energy production depends largely on metabolism of glycogen via aerobic pathways.⁷⁶ Within a few minutes, glucose and FFA concentrations rise in the blood and following 20–30% glycogen depletion, there is a shift towards β -oxidation of FFA.⁷⁹ With higher energy demands, the muscle

ATP/ADP ratio decreases, providing a stimulus for energy production via anaerobic mechanisms. The activity of the key regulatory glycolytic enzyme phosphofructokinase increases, causing greater production of pyruvate via glycolysis. The point where the availability of oxygen becomes a limiting factor in oxidative phosphorylation is reflected by partial reoxidation of NADH_2 , as more and more pyruvate is converted to lactate. As exercise intensity increases, the anaerobic pathway supplies a greater proportion of energy. The point when the increased rate of lactate production can be detected in the plasma is known as the anaerobic threshold. This threshold varies and depends on several factors including the muscle's fiber-type composition and the level of fitness. Furthermore, diet plays an additional role; for example, a fat-rich diet promotes oxidative energy production via FFAs,⁸⁰ thereby increasing the oxidative capacity of muscle^{81,82} and sparing glycogen.⁸³ However, other substrates also influence the pathways employed during energy production; for instance, energy production can be steered towards that derived from glucose by the provision of additional glucose during exercise.⁸⁴

Muscle heterogeneity

The ability of muscle tissue to perform efficiently in spite of very different types of exercise of varying intensity and duration is mediated partly by overall nervous control. However, it is significantly enhanced by the heterogeneous fiber type composition of each muscle.

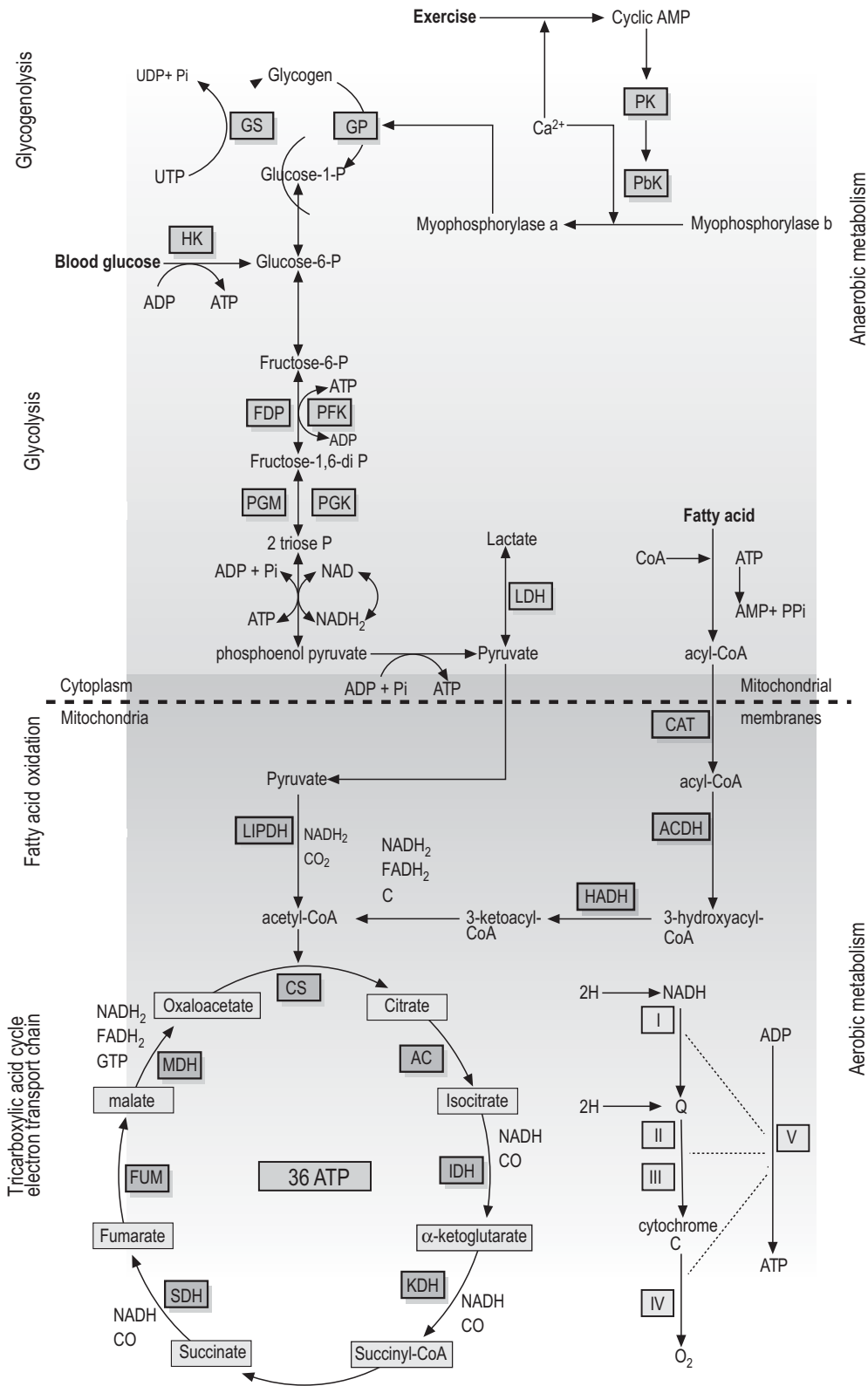


Fig. 2.1.17

Diagram summarizing the integration of metabolic pathways in muscle cells. Abbreviations: AC, aconitase; ACDH, acyl-CoA dehydrogenase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CAT, carnitine acyltransferase; CoA, coenzyme A; CS, citrate synthase; FAD, flavin adenine dinucleotide; FDP, fructose diphosphatase; FUM, fumarase; GP, glycogen phosphorylase; GS, glycogen synthetase; GTP, guanosine triphosphate; HADH, 3-hydroxyacyl-CoA dehydrogenase; HK, hexokinase; I, complex I; II, complex II; III, complex III; IDH, isocitrate dehydrogenase; IV, complex IV; KDH, ketoglutarate dehydrogenase; LDH, lactate dehydrogenase; LIPDH, lipoamide dehydrogenase; MDH, malate dehydrogenase; NAD, nicotinamide adenine dinucleotide; PbK, phosphorylase b kinase; PFK, phosphofructokinase; PGK, phosphoglyceratekinase; PGM, phosphoglyceratemutase; Pi, phosphate; PK, protein kinase; PPi, pyrophosphate; SDH, succinate dehydrogenase; V, complex V; UDP, uridine diphosphate; UTP, uridine triphosphate.

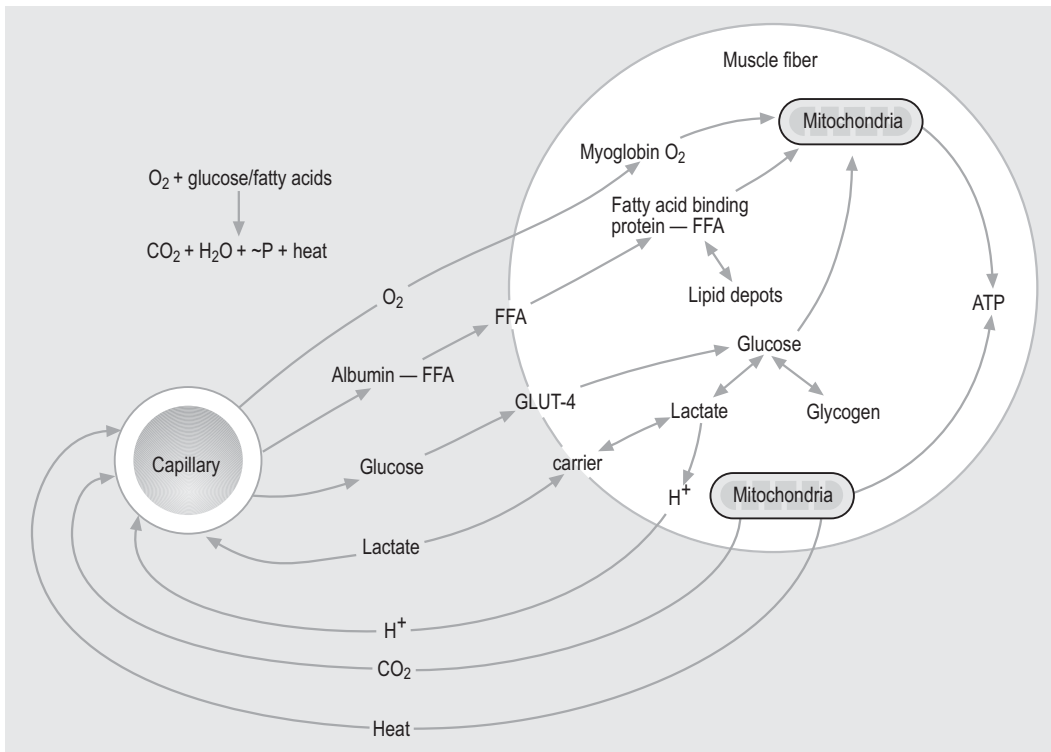
**Fig. 2.1.18**

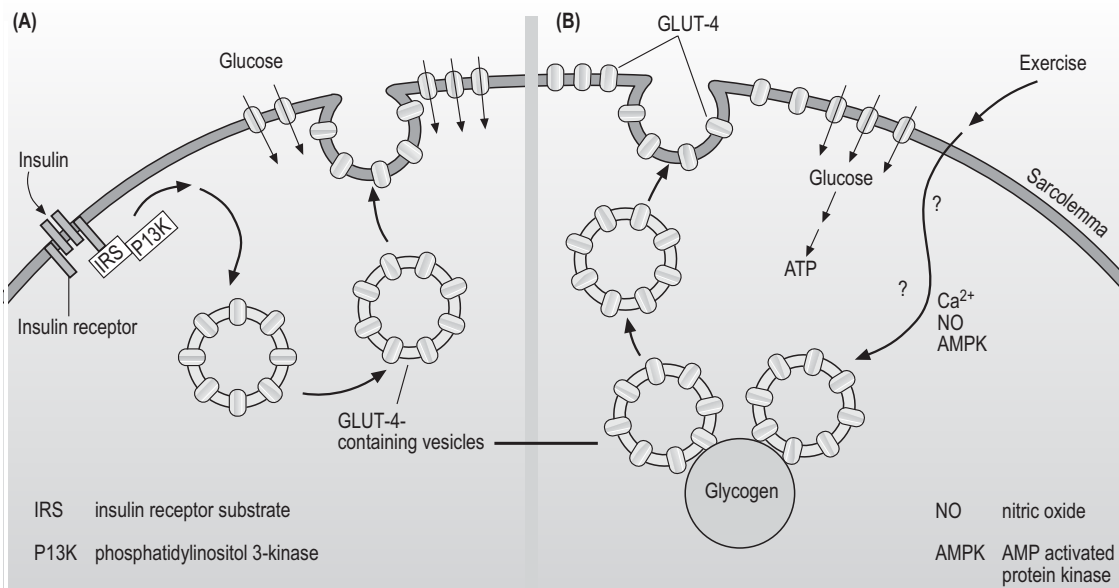
Diagram of intermediary steps involved in the transfer of substrates to and from skeletal muscle fibers. FFA, free fatty acids; GLUT-4, glucose transport protein 4. (Adapted from Booth & Baldwin.⁷⁷)

Muscle fiber types

Fiber type differentiation

There are important differences in the morphologic, physiologic, and biochemical properties of fibers both within and between muscles. These differences form the basis for the classification of fiber types. Better understanding of the expression patterns of groups of proteins within individual fibers has allowed refinement of fiber-type classification in recent years. For

example, myofibrillar proteins exist as different isoforms encoded by separate genes that are expressed in a myofiber type-specific and coordinated manner.⁸⁵ Fiber types can best be differentiated by analyzing the specific myosin heavy chain (MyHC) isoform(s) expressed by each fiber, since MyHC composition closely reflects each fiber's phenotype.²⁴ Three MyHC isoforms have been characterized in adult equine skeletal muscles at the protein,³⁸ mRNA,³⁹ and cDNA⁴⁰ levels; they are designated as types I, IIA, and IIX³⁸ (or

**Fig. 2.1.19**

Diagrammatic representation of insulin-dependent translocation of vesicle-bound GLUT-4 receptors from the cytoplasm to the sarcolemma (A) and the similar translocation of GLUT-4 receptors in response to exercise (B).

IID)³⁵ (henceforth IIX). The differential distribution of these MyHCs defines three pure fiber types containing a single isoform (types I, IIA, and IIX) and two hybrid fiber types coexpressing two isoforms (I+IIA and IIA + IIX (IIAX)) (Fig. 2.1.20). Hybrid IIAX fibers exist in equine locomotor muscles in significant numbers,^{35,36} although the coexpression of IIA and IIX MyHCs at the protein level seems not to be reflected by cotranscription of the corresponding genes (Fig. 2.1.21).⁴¹ This suggests that hybrids may represent fibers that are undergoing committed fiber type switching, towards the type corresponding to the mRNA currently being expressed, and that this conversion occurs in equine muscle not subjected to any specific training stimulus.⁴¹ Recent studies have demonstrated either minimal (less than 0.6%)⁸⁶ or no expression of the MyHC-IIB isoform in the horse;^{38–41} hence the fibers classified as type IIB in earlier studies are now more appropriately classified as type IIX. It remains unclear why the MyHC-IIB gene is only minimally (or not) expressed in various ungulates,⁴⁰ although it may be related to body size and muscle fiber length. As a muscle's maximum contraction velocity is proportional to the number of sarcomeres in series² and the MyHC-IIB isoform exhibits the fastest shortening velocity,⁸⁵ the

expression of this isoform in the very long muscle fibers of the horse would, in theory, result in an extremely high contraction velocity. Nevertheless, some recent studies have demonstrated the expression of this isoform in the skeletal musculature of some large mammals (pig and llama),^{87,88} indicating that the expression (or lack) of this isoform is not only a reflection of body size.

Muscle fiber type properties

Certain important differences between the various equine skeletal muscle fiber types are illustrated in Figure 2.1.22 and summarized in Table 2.1.1. When studied in combination, these differences allow more objective delineation (Fig. 2.1.23) and represent the considerable interdependence of contractile, metabolic, and morphologic features. Type I fibers have a MyHC isoform that hydrolyzes ATP slowly, resulting in a slow crossbridge cycle. These fibers have a small cross-sectional area, a high number of capillaries and high oxidative capacity. However, their glycolytic capacity and glycogen content are relatively low. Together, these properties make type I fibers highly efficient and economical in producing slow repetitive movements and sustaining isometric force, but not

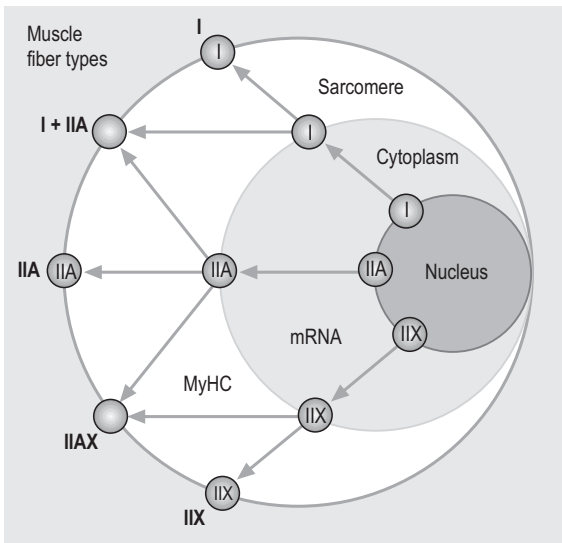


Fig. 2.1.20

Diagram illustrating the relationship between muscle fiber phenotype and the regulation of gene expression for the separate myosin heavy chain isoforms in adult horse skeletal muscle. Horses only express three myosin heavy chain isoforms: one slow or type I and two fast termed type IIA and type IIX. The differential expression of these isoforms defines three major fiber types, each containing a single isoform (i.e. types I, IIA, and IIX) and two intermediate hybrid fiber populations containing either slow and fast IIA isoforms or the two fast isoforms.

significant power generators. In contrast, type II fibers have MyHC isoforms that create fast crossbridge cycling and therefore develop force rapidly. Within the type II group, type IIX fibers have a maximal velocity of shortening that is three times higher than that of IIA fibers.¹⁸ Hence, IIX fibers are adapted for high power outputs for a limited time because they have a low oxidative capacity and limited oxygen availability (as reflected by their large cross-sectional area and relatively low capillary supply). Type IIA fibers, however, have a considerable number of both capillaries and mitochondria and rely on glycolytic and oxidative metabolism; they are therefore able to sustain high power outputs for longer than IIX fibers. Hybrid IIX fibers are intermediate in their properties.²⁴ Although they are classified according to the MyHC composition, it is important to remember that other protein isoforms vary, each closely correlating with the fiber's function and with one another. Fur-

thermore, fibers also differ with respect to other factors, such as the availability of high-energy phosphate,⁹⁰ GLUT-4 protein expression (Fig. 2.1.24),⁷⁷ the calcium sensitivity of force production,¹⁹ Ca^{2+} -ATPase content,⁹¹ carnosine and taurine contents,^{92–94} protein kinase C isoforms,⁹⁵ and expression of splice variants of neuronal nitric oxide synthase.⁹⁶

Muscle fiber recruitment

Although muscle can be separated into individual fiber types, the basic functional unit of skeletal muscle remains the motor unit (see section above on general muscle physiology). Motor units are commonly classified according to the MyHC profile of their constituent fibers (hence I, IIA, and IIX; Fig. 2.1.25). This is possible because fibers within a single motor unit show relatively homogeneous, although not identical, biochemical and histochemical properties.⁹⁷ Although the motor unit's structure, function, and hence role in motor control have been studied extensively in experimental animals, they have not been widely studied in larger mammals. However, specific muscle fiber type recruitment has been examined in horses by observing glycogen depletion patterns during and after exercise of varying intensity and duration (Fig. 2.1.26).⁵ In horses, it appears that motor units are selectively recruited in a specific pattern that changes according to the gait, and to the intensity and duration of exercise. For the maintenance of posture, only type I motor units are recruited. As intensity and duration of exercise increase, further motor units are recruited, in the rank order: I → IIA → IIX → IIX. Type IIX motor units are only recruited at near-maximal exercise intensity (sprint and jumping) and during extremely prolonged submaximal exercise.⁷⁶

Muscle fiber type distribution between and within muscles

In horses, muscle fiber types are spatially distributed according to the general checkerboard pattern present in mixed muscles of many other species. However, this pattern is never completely random, since fast-glycolytic type IIX fibers are more common along the rim than in the center of individual fascicles (see Fig. 2.1.3A).⁹⁸ Fiber-type composition varies extensively between muscles depending largely on function.⁹⁹ For example, significant components of the forelimb musculature consist of postural type I fibers, while propulsive muscles of the hindlimb contain a high proportion of fast-twitch type II fibers (Fig. 2.1.27A,B). Variation is also seen between muscles

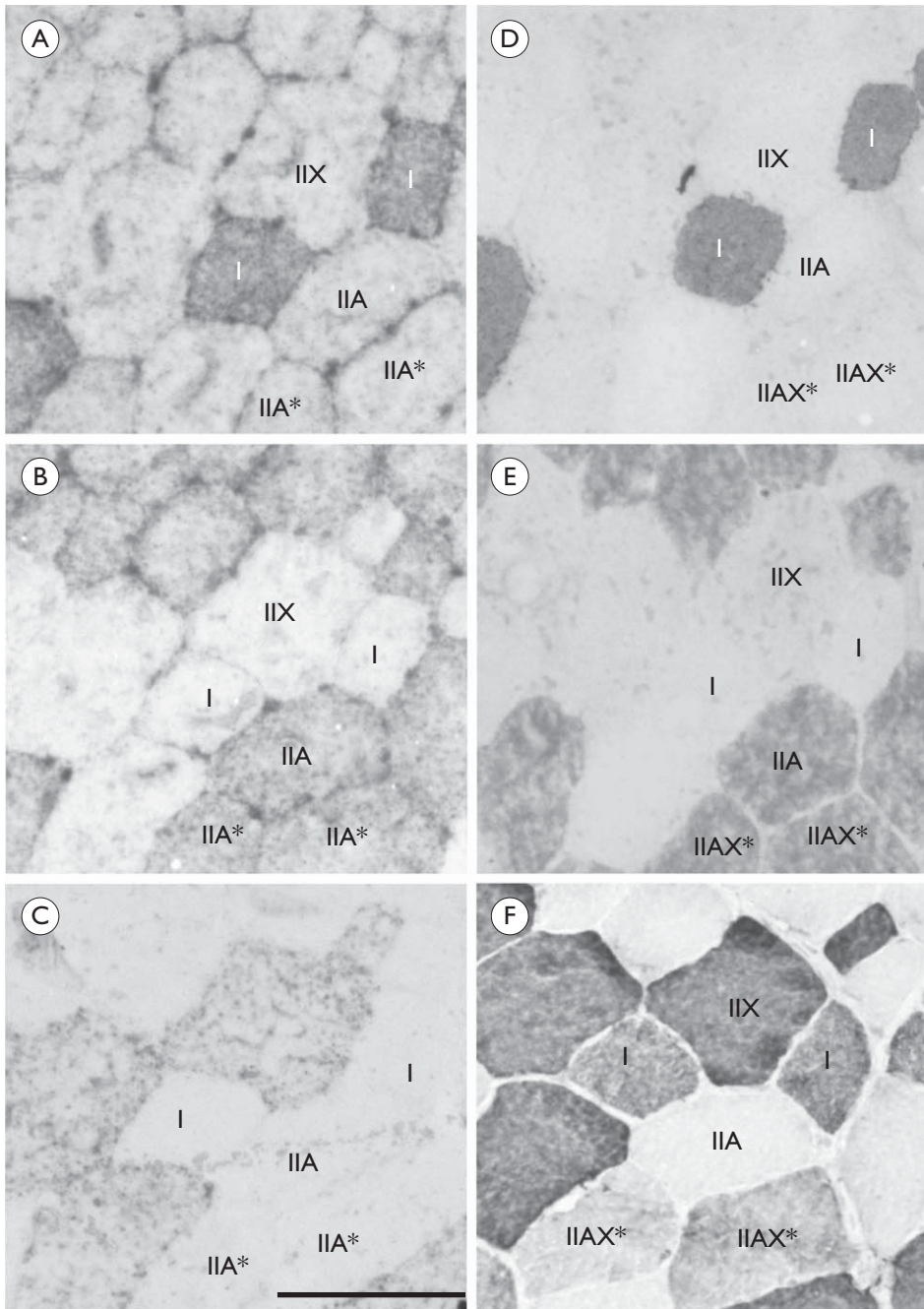


Fig. 2.1.21

Serial cryosections of adult untrained *M. gluteus medius* showing identification of myofiber types by in situ hybridization using non-radioactive MyHC mRNA probes specific for type I (A), type IIA (B), and type IIX (C) isoforms; and immunohistochemistry with a number of monoclonal antibodies to specific MyHC isoproteins: 219 (D, anti-MyHC-I), 333 (E, anti-MyHC-IIA), and 412 (F, anti-MyHCs I plus IIX). Fiber types are identified according to the mRNA expression (A–C) or according to the protein expression (D–F). Two hybrid IIA fibers at the protein level but expressing only type IIA mRNA are labelled with asterisks. Calibration bar, 100 μm. (Reproduced, with permission, from Eizema K, van den Burg MM, de Jonge HW, et al. Myosin heavy chain isoforms in equine gluteus medius muscle: comparison of mRNA and protein expression profiles. *J Histochem Cytochem* 2005; 53:1383–1390.)

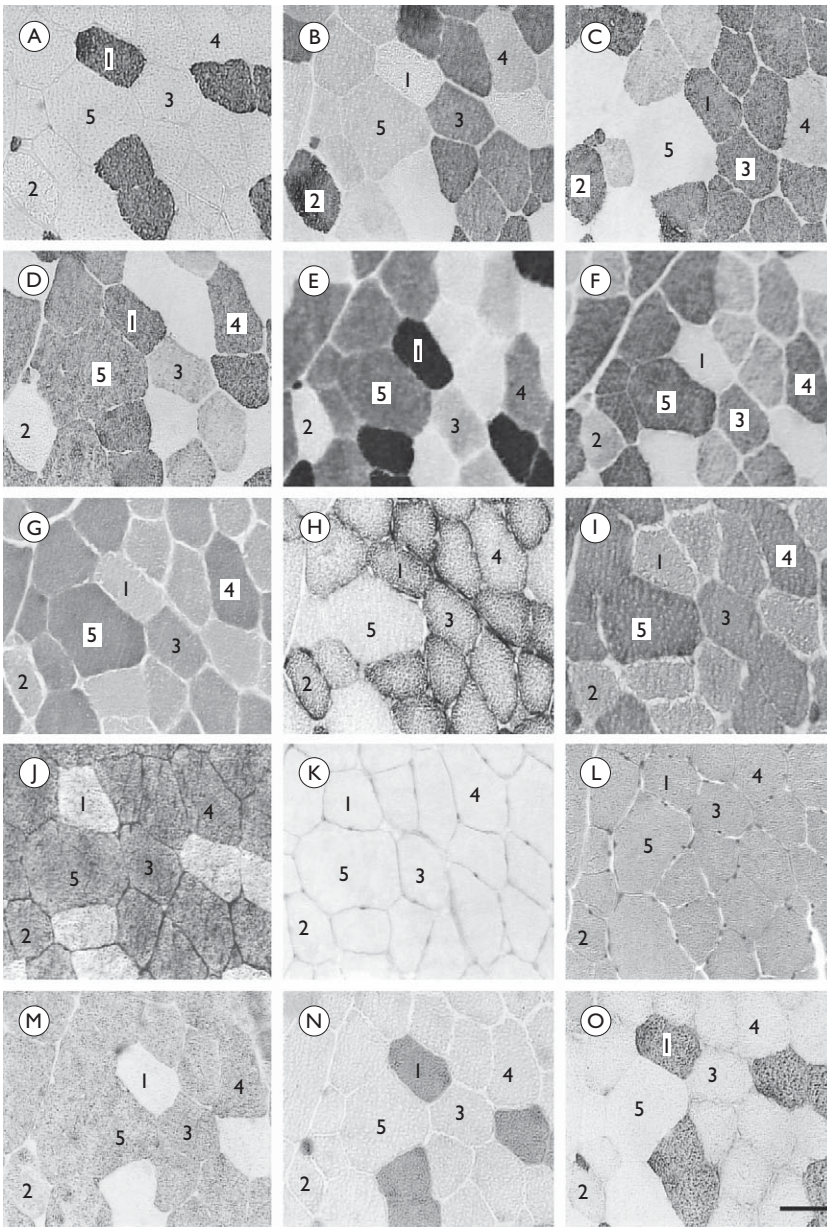
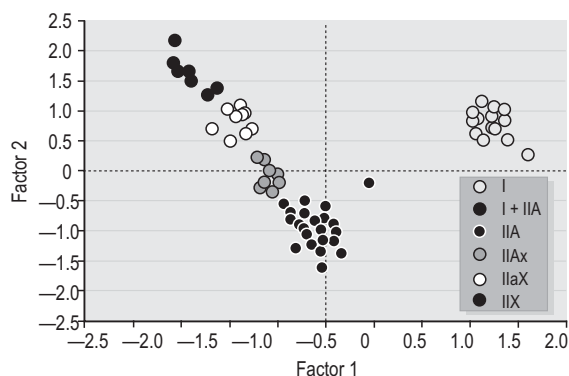


Fig. 2.1.22

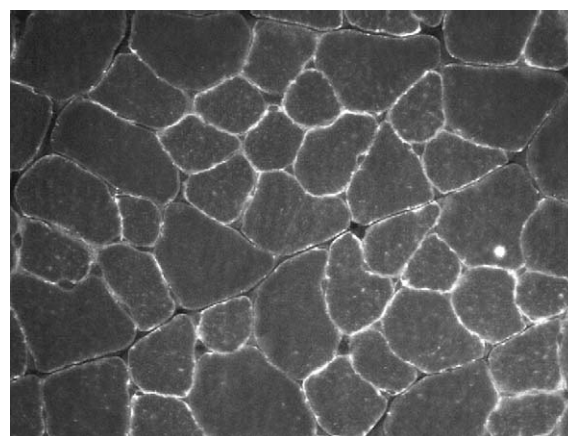
Serial cryosections of adult horse *M. gluteus medius* stained by immunohistochemistry, enzyme histochemistry, and histology. (A–D) Sections were stained with a number of monoclonal antibodies against specific myosin heavy chain (MyHC) isoforms: BA-D5 (A, anti-MyHC-b/slow), SC-71 (B, anti-MyHC-IIA), BF-35 (C, anti-MyHCs b/slow and IIA), and S5-8H2 (D, anti-MyHCs b/slow and IID/X). (E–G) Sections were assayed for myofibrillar actomyosin adenosine triphosphatase activity after acid (pH 4.4, E) and alkaline (pH 10.45, F) pre-incubations, and by Blanco and Sieck's quantitative histochemical procedure (G).⁸⁹ (H–I) Sections assayed for succinate dehydrogenase and (H), glycerol-3-phosphate dehydrogenase activities (I) and periodic acid–Schiff (PAS) for selective staining of glycogen (J). (K–L) PAS with α -amylase digestion, for visualizing capillaries (K), and hematoxylin and eosin for visualizing myonuclei (L). (M–O) Sections were stained by immunohistochemistry with monoclonal antibodies to SR Ca^{2+} -ATPase (SERCA) isoforms and phospholamban: CaF2-5D2 (M, anti-SERCA1a), MA3-910 (N, anti-SERCA2a) and 05-205 (O, antiphospholamban). The fibers labeled 1, 2, 3, 4, and 5 are types I, IIA, IIAx, IIX, and IIX, respectively. Calibration bar, 50 μm .

Table 2.1.1 Quantitative fiber type features of equine skeletal muscle¹

	Muscle fiber types ²				
	I	IIA	IIAx	IIaX	IIX
No. of fibers	51	80	25	25	27
Anti-MyHC monoclonal antibodies³					
BA-D5 (OD)	0.60 ± 0.02 b		0.34 ± 0.02 a		
SC-71 (OD)	0.30 ± 0.02 a	0.52 ± 0.03 d	0.46 ± 0.02 c	0.34 ± 0.01 b	0.34 ± 0.01 b
BF-35 (OD)	0.47 ± 0.03 c	0.49 ± 0.03 d	0.46 ± 0.03 cd	0.36 ± 0.02 b	0.30 ± 0.02 a
S5-8H2 (OD)	0.44 ± 0.02 d	0.33 ± 0.01 a	0.37 ± 0.02 b	0.41 ± 0.01 c	0.41 ± 0.01 c
Myofibrillar ATPase activity⁴					
Ac-mATPase (OD)	0.77 ± 0.02 d	0.28 ± 0.03 a	0.34 ± 0.03 b	0.47 ± 0.02 c	0.50 ± 0.02 c
Alk-mATPase (OD)	0.27 ± 0.02 a	0.37 ± 0.03 b	0.46 ± 0.04 c	0.52 ± 0.02 d	0.54 ± 0.02 d
Qu-mATPase (OD/min)	0.30 ± 0.02 a	0.39 ± 0.02 b	0.42 ± 0.01 c	0.45 ± 0.01 d	0.51 ± 0.01 e
Metabolic properties⁵					
SDH (OD/min)	0.49 ± 0.02 d	0.46 ± 0.03 c	0.37 ± 0.02 b	0.34 ± 0.02 b	0.24 ± 0.03 a
GPD (OD/min)	0.33 ± 0.02 a	0.36 ± 0.03 b	0.41 ± 0.01 c	0.46 ± 0.01 d	0.47 ± 0.01 d
PAS (OD)	0.34 ± 0.02 a		0.45 ± 0.03 b		
Fiber size, capillaries, and myonuclei⁶					
CSA (μm ²)	3124 ± 723 a	3339 ± 763 b	4623 ± 807 c	5039 ± 1293 c	7635 ± 2930 d
cap/10 ³ μm ²	2.06 ± 0.94 b	1.96 ± 0.57 b	1.60 ± 0.41 ab	1.38 ± 0.21 a	1.23 ± 1.07 a
nuc/10 ³ μm ²	2.26 ± 0.65 b	2.32 ± 1.02 b	1.65 ± 0.36 ab	1.44 ± 0.78 a	1.11 ± 0.70 a

**Fig. 2.1.23**

Principal component analysis of muscle fiber type features in a representative sample ($n = 82$ fibers) to show graphically the spatial discrimination of myosin heavy chain (MyHC)-based fiber types. This scatter plot shows the spatial distribution of a set of equine muscle fibers upon the basis of muscle variables included in Table 2.1.1; this analysis resulted in an optimal discrimination (100%) of all muscle fiber types; Factor 1 axis discriminates between type I (right) and type II (left) fibers, whereas Factor 2 axis discriminates type IIA (bottom) and IIX (top) fibers.

**Fig. 2.1.24**

Transverse section of rat extensor digitorum muscle samples labeled by immunohistochemistry with a monoclonal antibody to GLUT-4 (glucose transport protein). Note the higher intensity of staining in the periphery of the smaller fibers (presumably types I and IIA) compared with the larger ones (presumably types IIX and IIB).

Table 2.1.1 Quantitative fiber type features of equine skeletal muscle¹—cont'd

	Muscle fiber types ²				
	I	IIA	IIAx	IlaX	IIX
No. of fibers	51	80	25	25	27
Anti-SERCA and PLB antibodies⁷					
CaF2-5D2 (OD)	0.33 ± 0.03 a		0.44 ± 0.03 b		
MA3-910 (OD)	0.48 ± 0.02 b		0.38 ± 0.03 a		
05-205 (OD)	0.54 ± 0.03 b		0.37 ± 0.02 a		

¹Values are mean ± SE. Within a row, means with different letters are statistically different ($P < 0.05$), where a expresses the lowest value and e the highest. In the absence of significant differences between type II fibers, values are presented as pooled means for all type II fibers ($n = 157$ fibers). OD = optical density.

²Hybrid I+IIA fibers are not considered in this analysis; IIAx = hybrid fibers with a predominant myosin heavy chain type IIA content; IlaX = hybrid fibers with a predominant myosin heavy chain type IIX isoform.

³See Figure 2.1.22's legend for origins and specificities of antibodies.

⁴Ac-mATPase = myofibrillar ATPase after acid (pH 4.45) pre-incubation; Alk-ATPase = myofibrillar ATPase after alkaline (pH 10.45) pre-incubation; Qu-mATPase = quantitative myofibrillar ATPase activity (pH 7.6).

⁵SDH = succinate dehydrogenase activity; GPD = glycerol-3-phosphate dehydrogenase activity; PAS = periodic acid-Schiff.

⁶CSA = cross-sectional area; cap = number of capillaries; nuc = nuclear number.

⁷SERCA = sarco(endo)plasmic reticulum Ca^{2+} -ATPase; PLB = phospholamban; see reference²⁰ for sources and specificities of these antibodies.

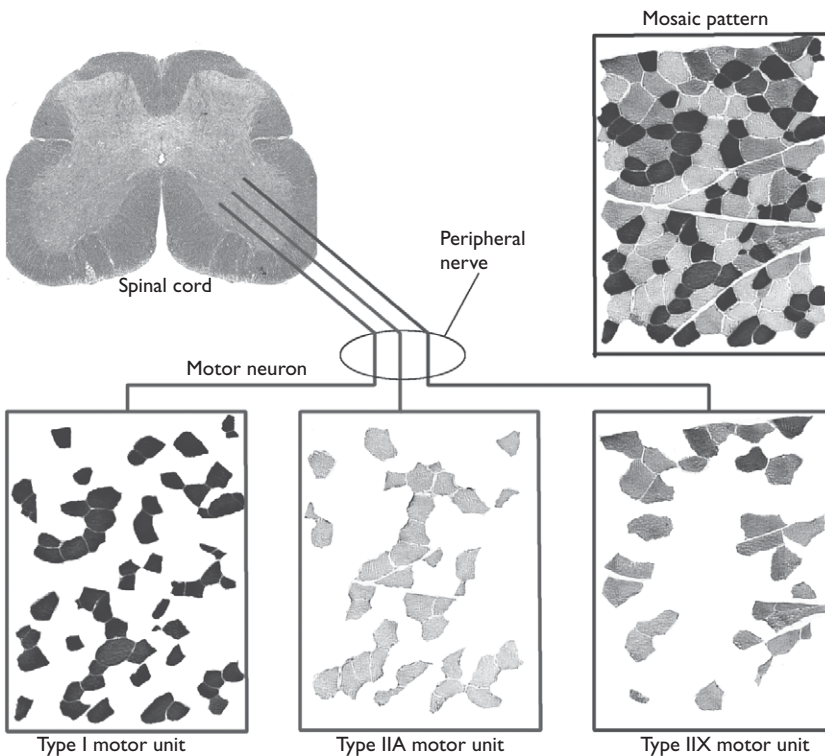
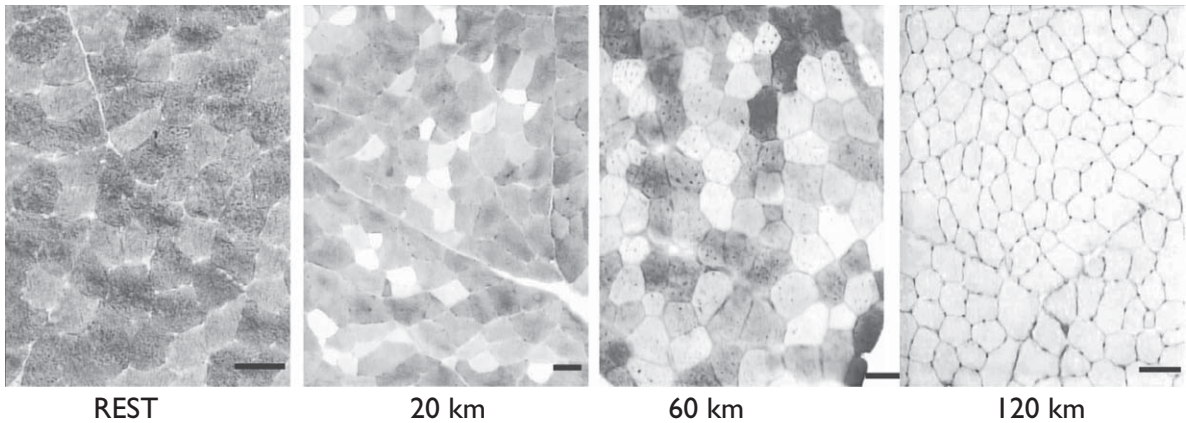
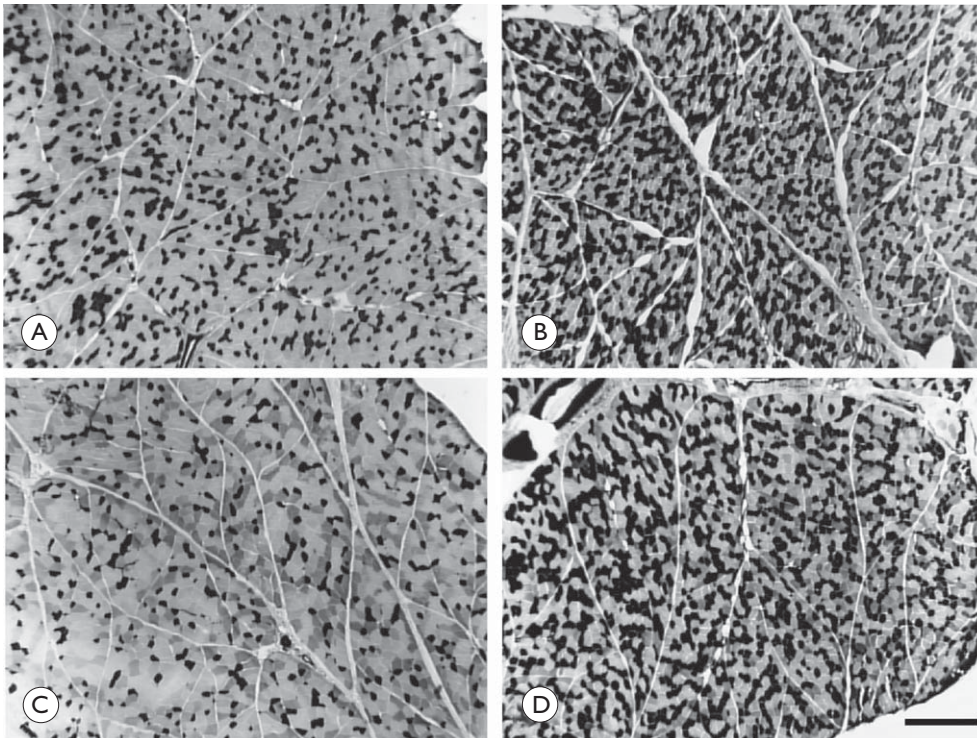
**Fig. 2.1.25**

Diagram showing the spatial distribution of muscle fibers integrating three different motor units classified according to myosin heavy chain isoform expression as types I, IIA, and IIX. Note how muscle fibers within each motor unit are dispersed between those of others producing the characteristic mosaic or checkerboard distribution seen in transverse section.

**Fig. 2.1.26**

Transverse sections of gluteus medius muscle biopsies (6 cm depth) to show muscle glycogen depletion patterns in a horse competing in an endurance ride of 120 km. Note the increase in the number of myofibers without glycogen when the intensity and duration of the exercise increase. Bars, 75 μ m.

**Fig. 2.1.27**

(A, B) Transverse sections stained for myofibrillar adenosine triphosphatase activity after acid pre-incubation (pH 4.4) demonstrating the differential muscle fiber type composition in two locomotor muscles with different functions: M. semitendinosus (A) vs M. rhomboideus cervicis (B). Note the higher percentage of type I fibers (black staining) and the lower proportion of types IIA (white) and IIX fibers (gray) in the rhomboideus muscle compared with the semitendinosus muscle. (C, D) Transverse sections stained with myofibrillar adenosine triphosphatase activity after acid pre-incubation (pH 4.4) from two muscle samples removed from the equine gluteus medius muscle at two different sampling depths: 2 cm (C) vs 8 cm (D). Note the higher percentage of type I fibers and the lower proportion of type IIX fibers in the deeper region of the muscle than in the superficial region. Scale bar, 1000 μ m.

belonging to the same synergic group. For example, most of the triceps muscle mass consists of type II fibers, but the medial head is composed of nearly all type I fibers.¹⁰⁰ Significant regional variation in fiber composition within a muscle has also been reported in several horse muscles.^{11,12,28,29,36,58,59,101} Most locomotory muscles have greater numbers of oxidative type I and type IIA fibers in the deep portions and a predominance of glycolytic type IIX in more superficial portions (Fig. 2.1.27C,D). This compartmentalization reflects the relationship between structure and function: the deeper regions appear best suited for posture maintenance and low-level, but longer duration muscular activity, whereas the more superficial regions are involved with short-duration, rapid propulsive force generation. In general, groups studying equine muscle physiology have formed conclusions based on data derived from a single biopsy sample site (see reference⁵ for review of relevant citations); however, it is apparent that this approach may be too simplistic. Several studies by the first author's group for instance, have demonstrated a greater muscular response to training in the deeper region of the M. gluteus medius compared with more superficial regions.^{102–104}

Relationship to performance

Some studies in horses have shown that performance is correlated with selected muscle characteristics (see reference⁵ for a review). Unsurprisingly, endurance capacity is correlated with (1) high percentages of type I and IIA fibers¹⁰⁵ and (2) high activities of oxidative enzymes,¹⁰⁶ whereas sprint capacity is correlated with high percentages of type II fibers.¹⁰⁷ It is therefore feasible to differentiate the endurance potential of horses based on the fiber type composition of certain muscles,¹⁰⁸ although different conclusions have been obtained in Thoroughbreds¹⁰¹ and trotters.^{109,110} Nevertheless, trotting speed is highly dependent on the ability of muscle to produce energy via anaerobic glycolysis.¹⁰⁹ Interestingly, some myofiber properties are correlated with kinematic profiles. For example, stride length and frequency are positively correlated with both the percentage of IIA fibers¹¹¹ and fiber size,¹¹² and the stance time of the stride is inversely correlated with the percentage of IIX fibers¹¹³ and fiber diameter.¹¹² Furthermore, certain primary muscular adaptations to training occur with concomitant modifications in the temporal characteristics of the trot.¹¹⁴

Control and regulation

Myogenic factors

Multiple factors, both myogenic and non-myogenic in origin, regulate the expression of proteins that comprise the various muscle-specific characteristics of each muscle fiber (Fig. 2.1.28) and, in combination, these factors also regulate the percentage of individual fiber types found within each muscle. During development and maturation (and regeneration following injury — see reference⁴⁷) these factors change, resulting in significant changes in protein expression. However, the myogenic lineage from which a muscle fiber develops generally defines its ultimate type.⁸⁵ All fibers during embryonic development express an embryonic MyHC isoform. At birth, some fibers are found to express type I MyHC; these are destined to become mature type I fibers in the adult. Other fibers express a neonatal MyHC isoform that is gradually replaced by either IIA or IIX, or both MyHCs; these subsequently become type II fibers in the adult. Horse skeletal muscle has also been found to express the α -cardiac MyHC isoform,³⁵ but its significance is unclear.

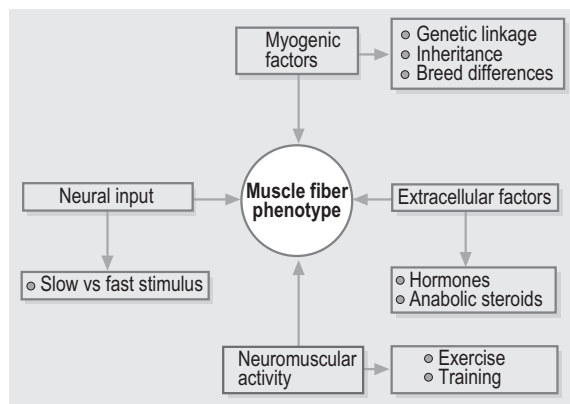


Fig. 2.1.28

Schematic diagram summarizing regulation of muscle fiber type. Fiber type is determined by a combination of four factors: (1) the myogenic lineage from which the muscle fiber developed; (2) the innervating motor neuron that regulates fiber type via activity pattern and basal activity and/or via other mediators such as neurotrophic factors; (3) activity levels affected by exercise and training; and (4) extracellular factors. The extracellular factors include hormones (e.g. thyroid hormone and growth hormone), growth factors (e.g. insulin-like growth factor), anabolic steroids, substrate availability (e.g. β -guanidinopropionic acid), and other unidentified factors (e.g. certain extracellular matrix proteins).

The influence of genetic factors on equine muscle fiber types is clearly illustrated by dramatic variations observed between different breeds (Fig. 2.1.29)^{99,115,116} and between separate bloodlines within the same breed.¹¹⁷ Furthermore, there is a tendency for fiber type ratios (type I : type II) to be inherited.^{107,118} During growth and maturation, muscle fibers change in their size and histochemical properties; there is a gradual conversion of fast to slow phenotype that is especially pronounced in the first year postpartum,¹¹⁹ but that may continue until about 6 years of age^{120–122} or older.¹²³ Hybrid fibers play an important role in this

process.¹²⁴ Interestingly, aged (20+ years) sedentary horses exhibit a shift in MyHC and metabolic profiles toward faster and more glycolytic phenotypes,^{125,126} which may explain some of the lower exercise capacity seen in the older horse.¹²⁷

Non-myogenic factors

In addition to the underlying myogenic lineage, additional factors influence muscle fiber phenotype. Muscle fibers are syncytial (multinucleated), with their myonuclei arranged peripherally throughout the length of

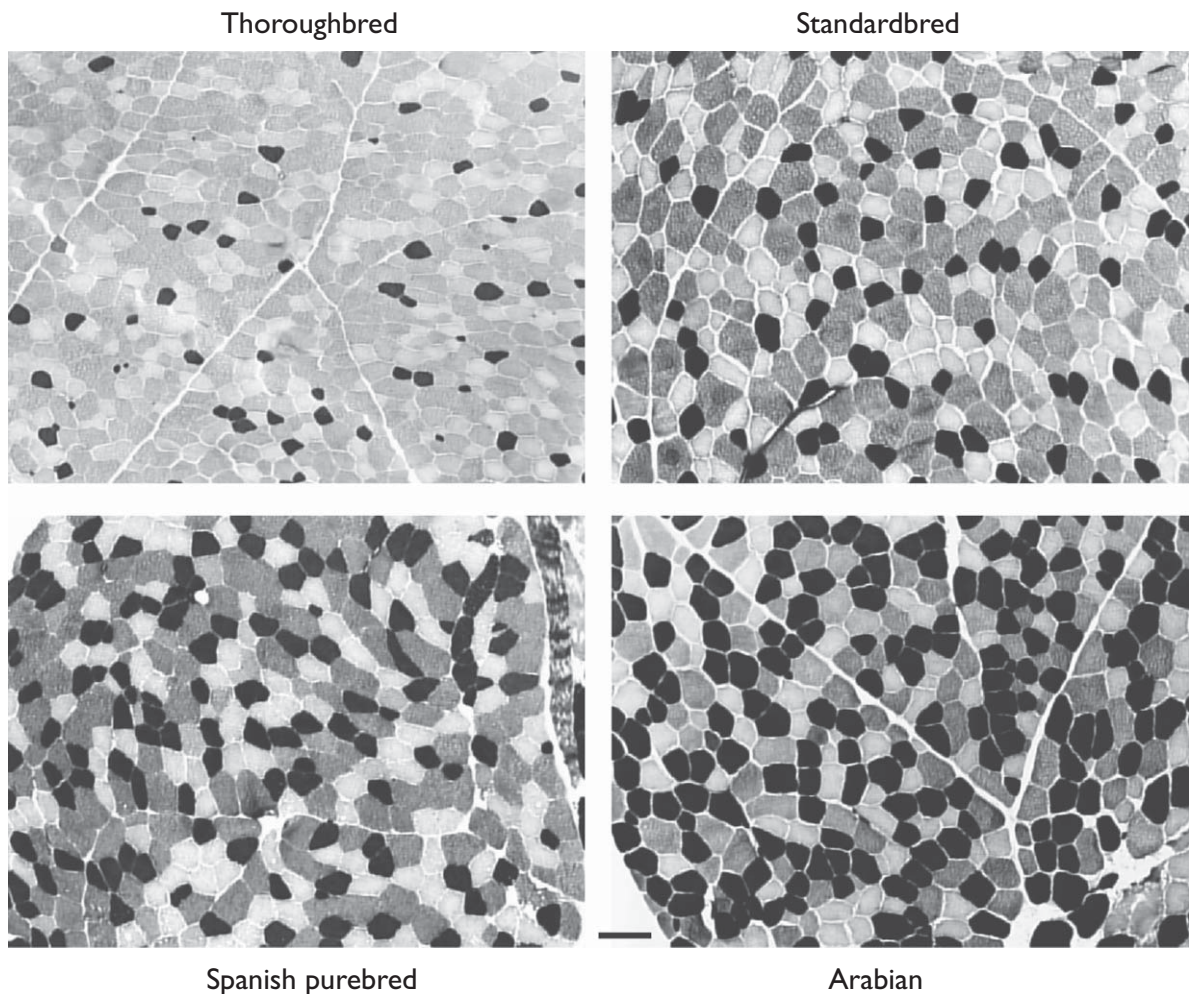
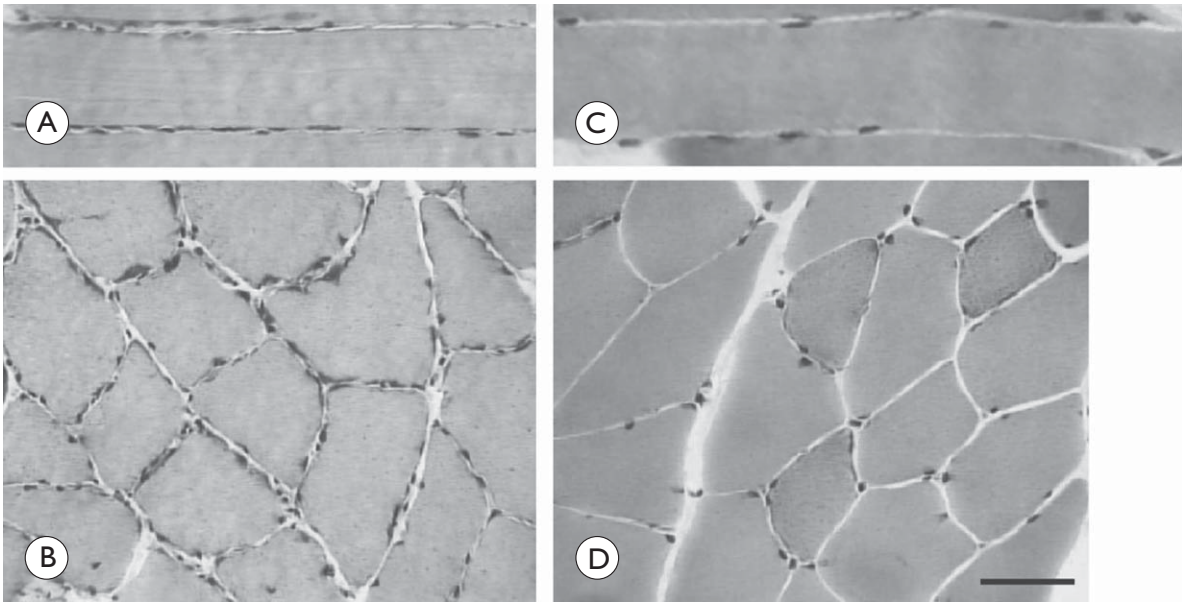


Fig. 2.1.29

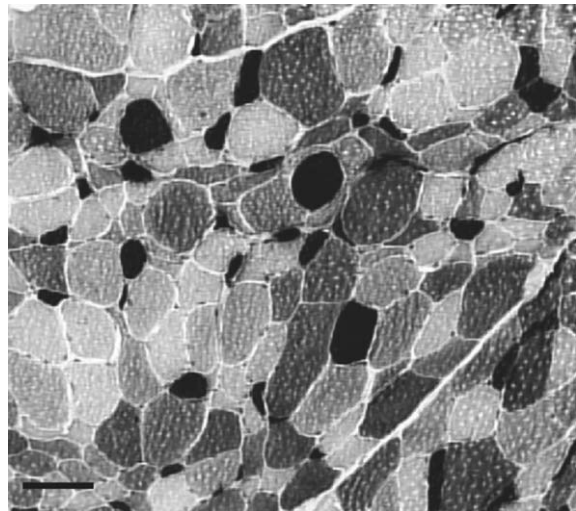
Transverse sections of *M. gluteus medius* specimens stained with myofibrillar adenosine triphosphatase after acid pre-incubation (pH 4.4), removed at the same depth, from four different breeds of athletic horse. Note how the percentage of type I (oxidative) fibers and the fiber size increase from the fastest breed (Thoroughbred) towards the slower, more endurance-suited breed (Arabian). Bar scale, 150 μm.

**Fig. 2.1.30**

Representative images of longitudinal (A and C) and transverse (B and D) sections stained with hematoxylin and eosin of a muscle containing predominantly slow fibers (type I) (*M. sacrocaudalis dorsalis medialis*, A and B) compared with a muscle containing predominantly fast-contracting (type II) fibers (superficial region of the *M. longissimus lumborum*, C and D). Note the higher myonuclear number in the fibers from the slow-contracting muscle compared with the fast-contracting muscle. Scale bar, 50 μm .

the fiber. The volume of cytoplasm associated with a single nucleus is known as the myonuclear domain.¹²⁸ Each individual nucleus therefore determines the expression of proteins within a particular cytoplasmic region. In horses, myonuclear domains of type I fibers are smaller than those of IIX fibers, but similar in size to those of IIA fibers (Fig. 2.1.30).²⁴ These observations have been related to the different activity patterns of the various fiber types;¹²⁹ the more active type I fibers have a higher rate of both protein synthesis and protein turnover than the less frequently recruited faster fiber types.

Neural input also has a significant influence on muscle fiber growth and type (fast/slow, glycolytic/oxidative) through altered regulation of gene expression.¹³⁰ This is convincingly demonstrated by dramatic changes observed in muscles following denervation (Figs 2.1.10, 2.1.31).¹³¹ Additional factors that are known to influence myofiber diversity include hormonal and drug (anabolic steroid)-induced changes,^{5,120–122} that may vary depending on the underlying fiber type.¹³² For example, chronic administration of clenbuterol, a β_2 -adrenoceptor agonist primarily used for treating some respiratory diseases in the horse, with and without exercise,

**Fig. 2.1.31**

Transverse section of a *M. vastus lateralis* muscle sample from a horse with denervation atrophy (femoral nerve paralysis) stained with myofibrillar adenosine triphosphatase after acid pre-incubation. Note selective angular atrophy of some type I (black) and type IIA (white) fibers, while other type I and IIA fibers presumably belonging to other motor units are of normal size. Bar, 50 μm .

results in a significant change in MyHC profile toward the faster phenotype,¹³³ although not affecting the Ca^{2+} -activated contractile characteristics of isolated myofibers.¹³⁴ Of particular interest, and as discussed in the sections that follow, neuromuscular (contractile) activity associated with exercise and training has a significant impact on fiber type adaptation and the expression of fiber-specific protein isoforms.

Muscular responses to exercise

When aerobic metabolism can meet energy demands (during submaximal exercise), oxygen uptake correlates with increasing speed.⁷⁸ However, the slope of the linear relationship may vary according to the load, incline, track surface, and ambient temperature.⁷⁸ At a certain point, energy demand outstrips oxygen uptake and the shortfall must be met by anaerobic metabolism.⁷⁸ Muscle fatigue may occur during either aerobic or anaerobic exercise; in the following sections, we consider the major metabolic changes that occur within muscle that are believed to contribute to the development of fatigue.

Aerobic exercise

Muscle and liver glycogenolysis starts to occur soon after the start of aerobic exercise. Glucose derived from the liver is subsequently transported into myofibers to join the glycolytic cascade via glucose-6-phosphate formation (see Fig. 2.1.17). However, although elevated glucose-6-phosphate concentrations have been detected after submaximal exercise in horses,¹³⁵ circulating epinephrine (adrenaline) released during exercise stimulates the release of FFAs from adipose tissue and/or liver stores, which partially inhibit glucose utilization during moderate-intensity exercise.¹³⁶ Nevertheless, during prolonged submaximal exercise, blood glucose may still account for up to 25% of the total energy output.⁷⁶ This reliance on glucose derived mainly from the liver results in an early sparing of muscle glycogen. As energy demands increase, higher rates of pyruvate oxidation tend to cause a further shift towards FFA β -oxidation. The overall effect is that muscle glycogenolysis declines over time during aerobic exercise, whereas FFA oxidation increases.⁷⁹

Although lipids are the predominant fuel utilized during prolonged submaximal exercise, fatigue occurs

long before the complete utilization of lipid stores.⁵ At submaximal workloads, fatigue has been associated with intramuscular glycogen depletion (Fig. 2.1.32)⁷⁶ because FFA oxidation cannot produce sufficient ATP without a source of pyruvate. During prolonged activity, glycogen depletion patterns occur in parallel with the progressive recruitment of fiber types, i.e. occurring initially in type I fibers, then in IIA, and finally in glycolytic IIX (see Fig. 2.1.26). This depletion (at least during submaximal long term exercise) affects the acid-soluble form of glycogen (macroglycogen) to a greater extent than the acid-insoluble form (proglycogen).¹³⁷ Muscular fatigue then does not occur at the same time in all fibers but in a progressive manner that results in gradual compromise to performance. Following exercise, glycogen replenishment (first the proglycogen pool, then macroglycogen)⁴¹ occurs in the reverse order (i.e. IIX $\rightarrow$ IIA $\rightarrow$ I) and may take up to 72 hours,¹³⁸ or sooner with the administration of dextrose¹³⁹ or nandrolone.¹⁴⁰ The apparently slower rate of glycogen synthesis after exercise in horses in comparison with other species may reflect the lack of increased GLUT-4 expression reported by some authors after grain feeding and exercise.^{141,142} Nevertheless, strenuous exercise causes an increase in total content of GLUT-4 protein in skeletal muscle: an increase that is attenuated by replenishing muscle glycogen stores through glucose infusion.¹⁴³ However, although glycogen depletion appears to play a major role in fatigue onset during aerobic exercise, a variety of other factors are also implicated, including AMP deamination, hyperthermia, dehydration, electrolyte depletion, and lack of motivation.^{89,144,145} The onset of fatigue itself may be hard to assess objectively but recent evidence suggests that electromyography may prove useful in the experimental setting.⁴³ Aerobic exercise in the horse is also associated with decreased serum branched-chain amino acid concentrations, but this decrease does not correspond to a measurable increase in total muscle amino acid concentrations.^{137,146} Further research is, however, required to evaluate amino acid metabolism better during exercise, as it appears to play an important role in both performance and recovery after exercise.

Additionally, increased oxygen utilization during exercise results in proportional increases in reactive oxygen species (ROS) production, potentially causing oxidative stress, a state where increased generation of ROS overwhelms body antioxidant protection, resulting in lipid, protein, and DNA damage. Information on exercise-induced oxidative stress in horses is limited.¹⁴⁷ In response to oxidative stress, cells synthesize heat

shock proteins in protective homeostatic responses.¹⁴⁸ However, a moderate bout of aerobic exercise in horses, though associated with mild muscle damage and potentially mild oxidative stress, failed to induce this defense mechanism,¹⁴⁹ perhaps because acidosis, rather than energy depletion, appears to be the instigator of this response after long-term moderate-intensity exercise.¹⁵⁰

Anaerobic exercise

The functional demands imposed by high-intensity exercise require the recruitment of most motor units within a given muscle, while intramuscular glycogen and blood glucose act as the predominant fuels to replenish ATP during anaerobic glycolysis. Some ATP is derived from the deamination of adenosine nucleotides; however, in contrast to aerobic metabolism, there is relatively little reliance on FFA oxidation. The effects of high-intensity exercise on horse skeletal muscle and development of fatigue have been comprehensively reviewed by Snow & Valberg.⁵ In recent years further research has confirmed many previous observations and provided new insight on mechanisms underlying the onset of fatigue.

Lactate accumulation and pH decline

Limitations imposed by oxidative metabolism result in more pyruvate (the end-product of glycolysis) being

converted to lactate rather than acetyl-CoA; in the process, NAD is used to regenerate more ATP. As a consequence, muscle lactate concentrations increase during anaerobic exercise.^{109,110,151–153} This rise is correlated with the proportion of type II fibers within muscles.¹⁵⁴ Initially, intracellular lactate accumulation is removed from the cell by active transport into the blood (Figs 2.1.18, 2.1.33).¹⁵⁵ Saturation of this mechanism results in a sudden exponential rise in intracellular lactate accumulation known as the anaerobic threshold, that generally occurs when the plasma lactate concentration reaches about 4 mmol/L.

The rise in intracellular lactate, together with free H^+ ions, significantly lowers the cytoplasmic pH,¹⁵⁷ a factor believed to be the major cause of fatigue during anaerobic exercise (see Fig. 2.1.32).⁷⁶ Muscle pH may decline to as low as 6.25–6.50 and lead to impairment of both structure and function; for example, abnormal mitochondrial and SR ultrastructure has been documented in horses that exhibit fatigue during maximal exercise.¹⁵⁸ Low pH also leads to dysfunction of the excitation–contraction coupling mechanism, through impairment of the SR calcium release via RYR1 channels, and decreased reuptake of calcium into the SR during relaxation.¹⁵⁶ Additionally, low pH inhibits the glycolytic enzyme phosphofructokinase, thereby diminishing ATP production.¹⁵⁴

A fall in cytoplasmic pH is partially overcome by a buffering system within myofibers (see Fig. 2.1.33).

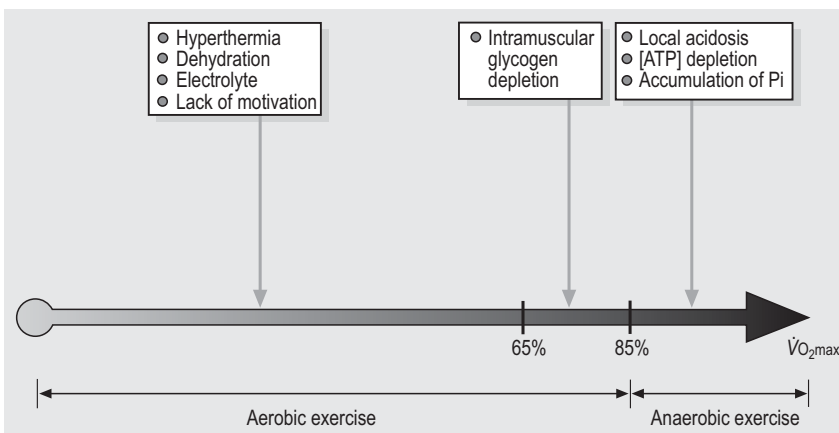
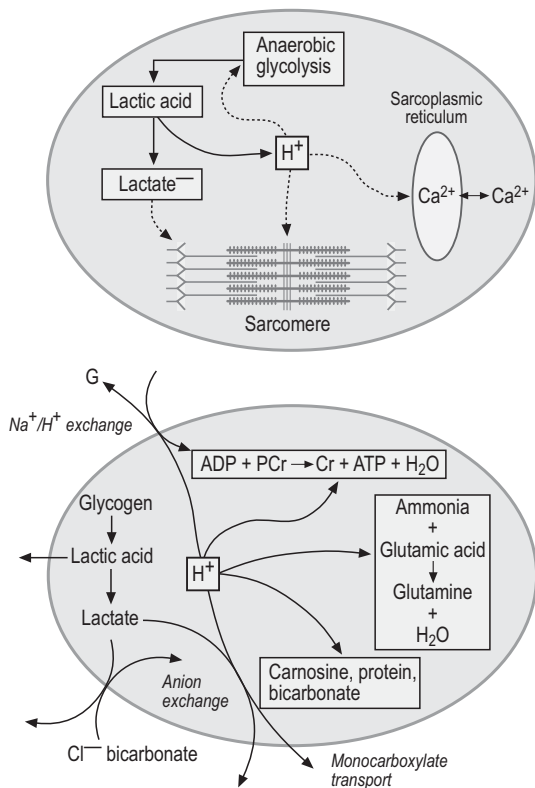


Fig. 2.1.32

Schematic diagram summarizing the main causes of fatigue during or after exercise of different intensity and duration. Intramuscular glycogen depletion is considered to be the main cause of fatigue during aerobic exercise (workloads between 65% and 85% of $\dot{V}O_{2\max}$). At lower exercise intensities, however, hyperthermia, fluid and electrolyte depletion, and poor motivation have been suggested to be the primary factors in initiating fatigue. Causes of fatigue during supramaximal anaerobic exercise include local acidosis, ATP depletion, and accumulation of pyrophosphates (Pi).

**Fig. 2.1.33**

Schematic diagrams illustrating intramuscular acidosis and buffering capacity of muscle (according to Hyyppä & Pösö).¹¹⁸ (Top) Performance inhibition (dotted arrows) caused by the accumulation of protons and lactate anions. (Bottom) Diagram summarizing the major regulatory mechanisms that control intramuscular pH (explained in reference¹¹⁸). PCr, phosphocreatine; Cr, creatine.

Dynamic buffering of H⁺ occurs during the hydrolysis of phosphocreatine in reactions catalyzed by creatine kinase and by glutamine synthetase; static physicochemical buffering is provided by various proteins, bicarbonate, inorganic phosphate, and carnosine.^{154,158} Race horses have a higher muscle buffering capacity than humans,¹⁵⁹ thought to be associated with their myofibers having high carnosine concentration.⁹³ Muscle carnosine content appears to be influenced by β-alanine bioavailability¹⁶¹ and is greatest in glycolytic IIX fibers.^{93,160}

Nucleotide depletion

Since initial observations by Snow and co-workers,¹⁶¹ numerous studies confirm a decline in muscle ATP concentration during anaerobic exercise,^{21,90,109,162}

suggesting that ATP regeneration may be insufficient to meet energy demands.⁹³ Depletion of muscle ATP concentration has also been implicated as a primary cause of fatigue during maximal effort in horses,⁷⁶ and a correlation between muscle nucleotide stores after racing and performance has recently been reported.⁹⁰ The fall in ATP occurs in parallel with a rise in IMP concentration,^{90,162} the latter being a particularly prominent response in equine muscle due to high underlying AMP deaminase activity.¹⁶³ High concentrations of IMP within muscle therefore act as a marker for depletion of total nucleotide stores, given that reamination of IMP to ADP and finally restoration of ATP is a slow process that may take up to an hour.¹⁶¹ ATP depletion and formation of IMP within muscles working maximally are closely correlated with the production of ammonia,^{110,151} uric acid¹⁶² and allantoin,⁷⁶ all of which may be detected in the plasma. Theoretically, low levels of ATP in some fibers would impair the optimal functioning of all ATP-dependent muscular processes. However, because nucleotide depletion occurs concurrently with a rise in muscle lactate and free H⁺ concentrations,⁹⁰ it is still unclear which of these mechanisms plays the more significant role in the development of fatigue.

Glycogen depletion

Muscle glycogen concentration declines rapidly during maximal exercise^{154,162} to an extent that varies between 30% and 50% depending on the number and frequency of exercise bouts.¹⁶¹ This degradation affects both proglycogen and macroglycogen.¹⁶⁴ Glycogen depletion occurs most rapidly in the glycolytic low oxidative IIX fibers simultaneously with lactate production.⁵ Although glycogen depletion is not considered to be a major factor contributing to fatigue during anaerobic exercise,⁷⁶ its depletion is associated with diminished anaerobic power generation and hence the capacity for high-intensity exercise.^{165,166}

Other muscular changes

Other factors that, though not necessarily contributing directly to fatigue, may result in reduced performance are reviewed by Snow & Valberg⁷⁶ and include increased intracellular potassium concentration,¹⁶⁷ a stoichiometrically altered proportion of free carnitine and acetylcarnitine,¹⁶⁸ increased formation of alanine from pyruvate,^{169,170} and significant changes in the free intracellular amino acid pool.¹⁷¹ Additional factors that are implicated include a reduction in calcium SR uptake through decreased Ca²⁺-ATPase activity,¹⁷²

increased Na^+/K^+ ATPase concentration,⁹¹ and a rise in muscle temperature that occurs during high-intensity exercise.¹⁵⁶ Finally, subclinical muscular disease may underlie poor performance in some horses.⁴⁷

Muscular response to training

Overview

The adaptation of equine skeletal muscle during training is largely mediated by the structural and functional plasticity of the myofibers. These long-term adaptations occur independently from the immediate or short-term physiologic responses to either aerobic or anaerobic exercise and are associated with altered rates and regulation of transcription of specific genes and consequently a change in the amount or specific isoform of proteins expressed within individual fibers.¹⁷³

Depending on the nature (type, frequency, intensity, and duration) of the stimulus (exercise training), the adaptive response can take the form of: (1) hypertrophy, when myofibers increase in size but otherwise retain their basal structural, physiologic, and biochemical properties; or (2) remodeling without hypertrophy, where myofibers do not enlarge but acquire markedly different enzymatic and structural characteristics, often accompanied by changes in the microvasculature; or (3) a mixed response, i.e. remodeling in conjunction with hypertrophy (Fig. 2.1.34). Furthermore, the modality and amplitude of the response depend significantly on the basal muscle profile before training¹⁷⁴ because the increased contractile activity that is associated with training induces a change towards slow and oxidative muscle profiles. Hence fast-twitch fibers (and therefore muscles that contain a higher proportion of glycolytic fibers) can show a relatively greater training adaptation than slow-twitch fibers. This response is particularly prominent in young, inactive¹⁷⁵ and aged, sedentary¹⁷⁶ horses,

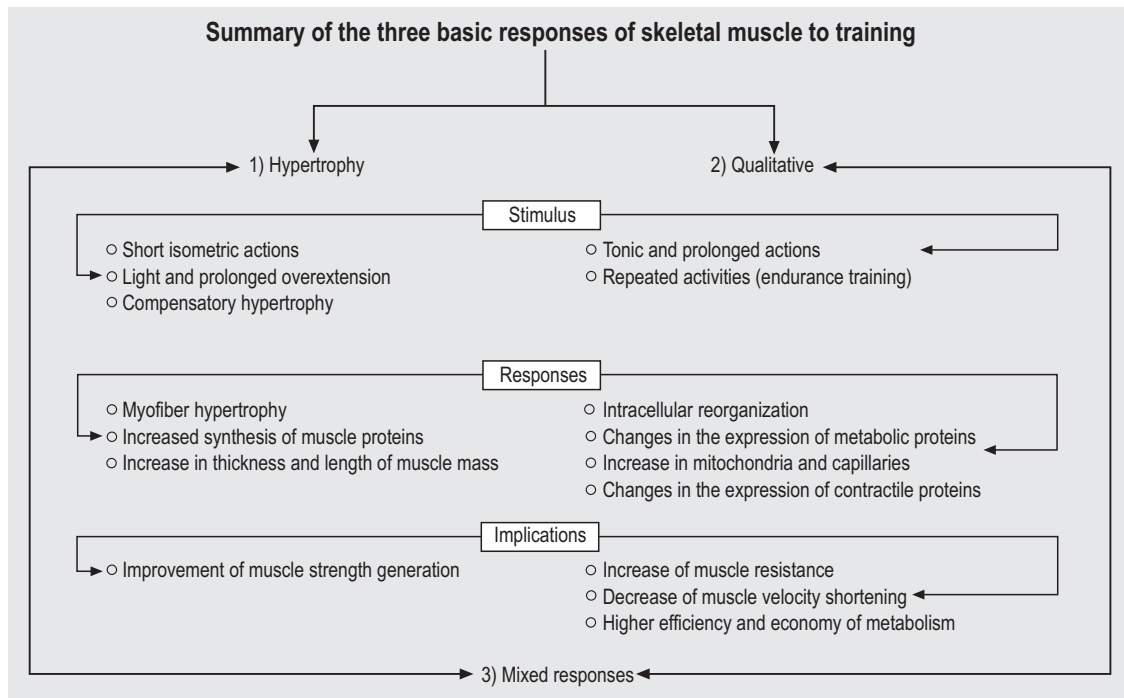


Fig. 2.1.34

Summary of the three basic responses of skeletal muscle to training: (1) hypertrophy, (2) remodeling without hypertrophy, and (3) remodeling with hypertrophy. Possible stimuli and the nature of the responses and physiological implications are indicated.

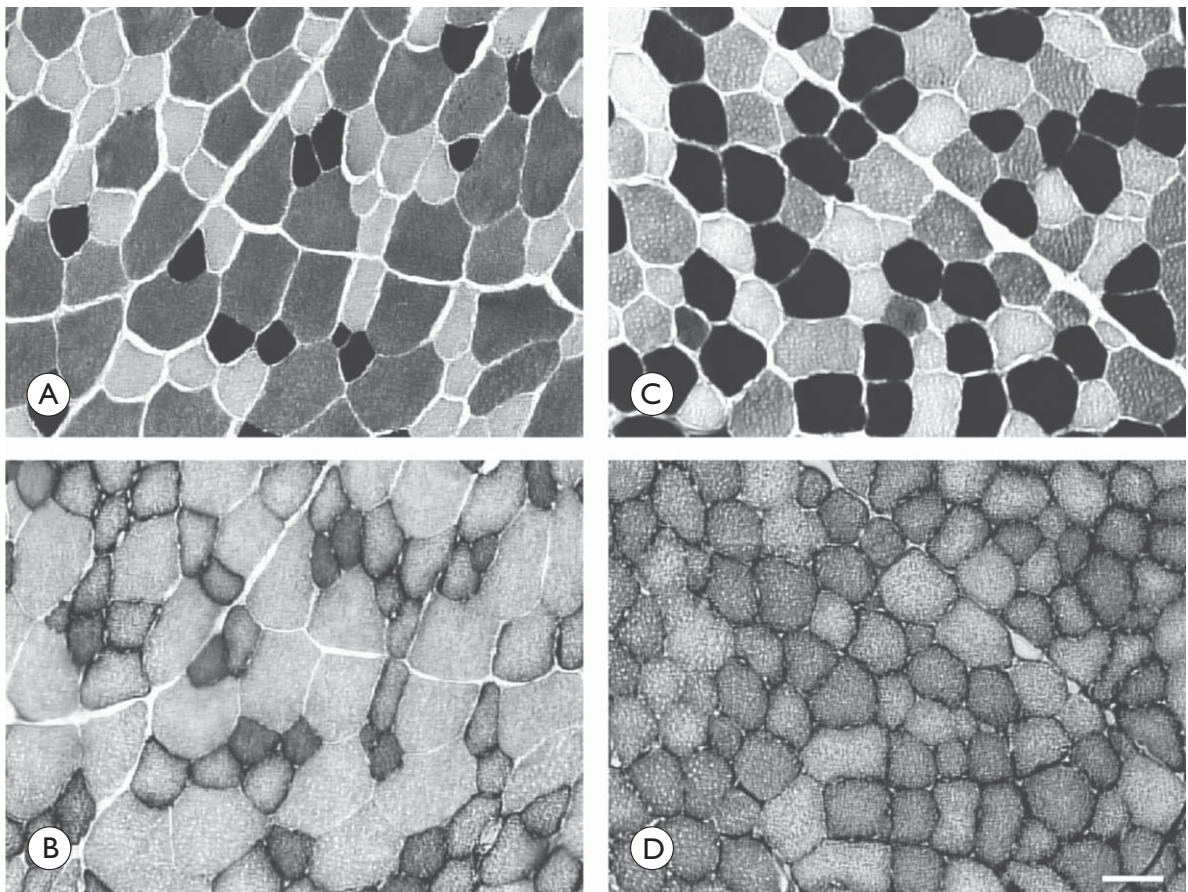


Fig. 2.1.35

Transverse serial sections of gluteus medius muscle biopsies stained with myofibrillar ATPase after acid pre-incubation at pH 4.45 (A and C) and succinate dehydrogenase (B and D) in a young adult (3-year-old) and untrained Andalusian horse (A and B), and in an adult and regularly trained (10-year-old) Andalusian horse (C and D). Note that the young animal has a lower proportion of type I fibers and a higher percentage of low-oxidative type IIX fibers than the adult horse; furthermore, differences in fiber size are much more pronounced in the young animal than in the adult. This therefore may explain the broader range of adaptive responses to training in young untrained horses than in adults or regularly trained animals. Bar, 50 μ m.

which have a high percentage of glycolytic (low oxidative) pure IIX fibers (Fig. 2.1.35A,B), in contrast to active mature horses, which have muscle fiber type profiles that are more oxidative (Fig. 2.1.35C,D). Although it can be hard to differentiate altered fiber properties caused by growth and training, specific training effects have been delineated in growing foals^{119,177} and young horses.^{103,104,175,178–181} Overall, muscular adaptations with training have important physiological implications that influence power generation, shortening velocity and resistance to fatigue.

Muscular adaptations to training

Muscle fiber size

The effects of training on equine muscle fiber size are still controversial. In general, the adaptive response of equine skeletal muscle to early and long-term exercise training takes the form of remodeling with minimal, if any, muscle fiber hypertrophy (see references⁵ and ¹⁸² for reviews). However, specific muscle fiber hypertrophy can be stimulated with bursts of high-resistance muscle activity^{103,183} and by prolonged stretch beyond

normal resting length.^{16,104} Weight bearing, as a form of progressive-resistance exercise training, has been investigated in ponies for improving strength, and resulted in increased muscle power, muscle size, and increased (though not statistically significant) type II fiber cross-sectional area, without parallel changes in MyHC composition.¹⁸³ Six months of conventional jump training in competitive showjumpers also induced a selective hypertrophic growth of type II fibers, with minimal switching between myofiber phenotypes.¹⁸⁴ Other longitudinal studies have also reported significant and early (less than 3 months) increases in the mean cross-sectional areas of type I and/or IIA fibers after training,^{16,86,102,103,114,175,185–187} a result also noted in horses less than a year of age.¹⁷⁷ A marked and generalized fiber hypertrophy (~50%) has recently been documented in endurance-trained Arabians performing moderate-intensity exercise (~80% of $\dot{V}O_{2\max}$) of long duration (50–80 minutes per day), 4 days/week for 3 months.¹⁸⁷ Even though a fiber transition occurs towards more oxidative fibers (IIX → IIA → I) with normally smaller cross-sectional area (see Table 2.1.1), the hypertrophy may represent either the new fibers retaining size characteristics of its previous type, or generalized increased protein production (or both). In contrast, other studies of Standardbreds and Thoroughbreds reveal minimal changes^{7,23,188,189} or even a reduction in fiber cross-sectional area.^{179,190–193} These observations are hard to reconcile with the prominent increase in muscle mass, especially in the hindquarters, generally observed in horses after most training programs.^{5,183} When considered together, the only explanation for an increase in muscle mass, despite either no change or a reduction in fiber size, is a parallel increase in the number of muscle fibers (hyperplasia), as previously demonstrated in humans.¹⁹⁴

Muscle fiber type transitions

Muscle fiber type distribution and MyHC composition are strongly influenced by training (Figs 2.1.36, 2.1.37). Studies on endurance training in horses have demonstrated (by myofibrillar ATPase histochemistry, immunohistochemistry and electrophoresis) increases in the fraction of type IIA fibers and a concomitant decrease in the proportion of IIX fibers.^{16,86,96,102–104,114,115,175,176,184,187,190,191,195–197} In addition, several endurance training studies in horses have reported fiber transitions beyond type IIA fibers: i.e. an increase in hybrid I+IIA fibers and pure type I fibers.^{7,102,104,198} Fiber type transitions during resis-

tance training appear to resemble qualitatively those observed in endurance training. Hence, strength training in horses has been shown to result in both increased IIA:IIX fiber ratio^{181,185} and, when training is long enough, the I:II fiber ratio.¹⁰³ Similarly, sprint training in horses results in increased numbers of type IIA and decreased numbers of type IIX fibers,¹⁹⁹ with a corresponding change in their respective MyHC content.^{86,193} In contrast to endurance and strength training, a specific decrease of type I fibers has been reported as an early, and probably transitory, response to high-intensity training.^{193,199}

When these various training studies are considered in combination, it is reasonable to assume that fiber type transitions occur in a graded and orderly sequential manner and typically change from faster, more glycolytic fibers to slower and more oxidative fiber types, i.e. IIX → IIAX → IIA → IIA+I → I.¹⁷⁴ A dose-response relationship between the duration (in total) of the training program and the magnitude of induced changes has recently been demonstrated at the molecular level.^{103,104} This relationship can be explained more readily in terms of a threshold for the type IIX → IIA transition during the early phase of training, and then a further threshold for the type IIA → type I transition. Thus, a single fiber is capable of a complete fast-to-slow transformation in response to a sufficiently long physiological training stimulus. Finally, although many reports describe the training response shown by muscle fibers in terms of the MyHC component, it is important to remember that many other protein isoforms of the sarcomere (such as the regulatory proteins of the thin filaments) and the calcium regulatory proteins of the SR change in parallel.²⁰⁰

Metabolic changes and increased capillary density

Perhaps the most commonly detected and earliest muscular adaptation to training is an increase in the activity of enzymes of aerobic metabolism, such as selected enzymes of the TCA cycle, the electron transport chain and fat oxidation.^{86,104,106,120,175–177,184,199,201,202} These changes are associated with increased mitochondrial and capillary densities (Fig. 2.1.38),^{16,102,113,197,203} the latter response promoting improved oxygen diffusion and more expeditious removal of waste products (such as CO₂).

The activities of key anaerobic enzymes, such as phosphofructokinase and lactate dehydrogenase, either do not change or decrease following train-

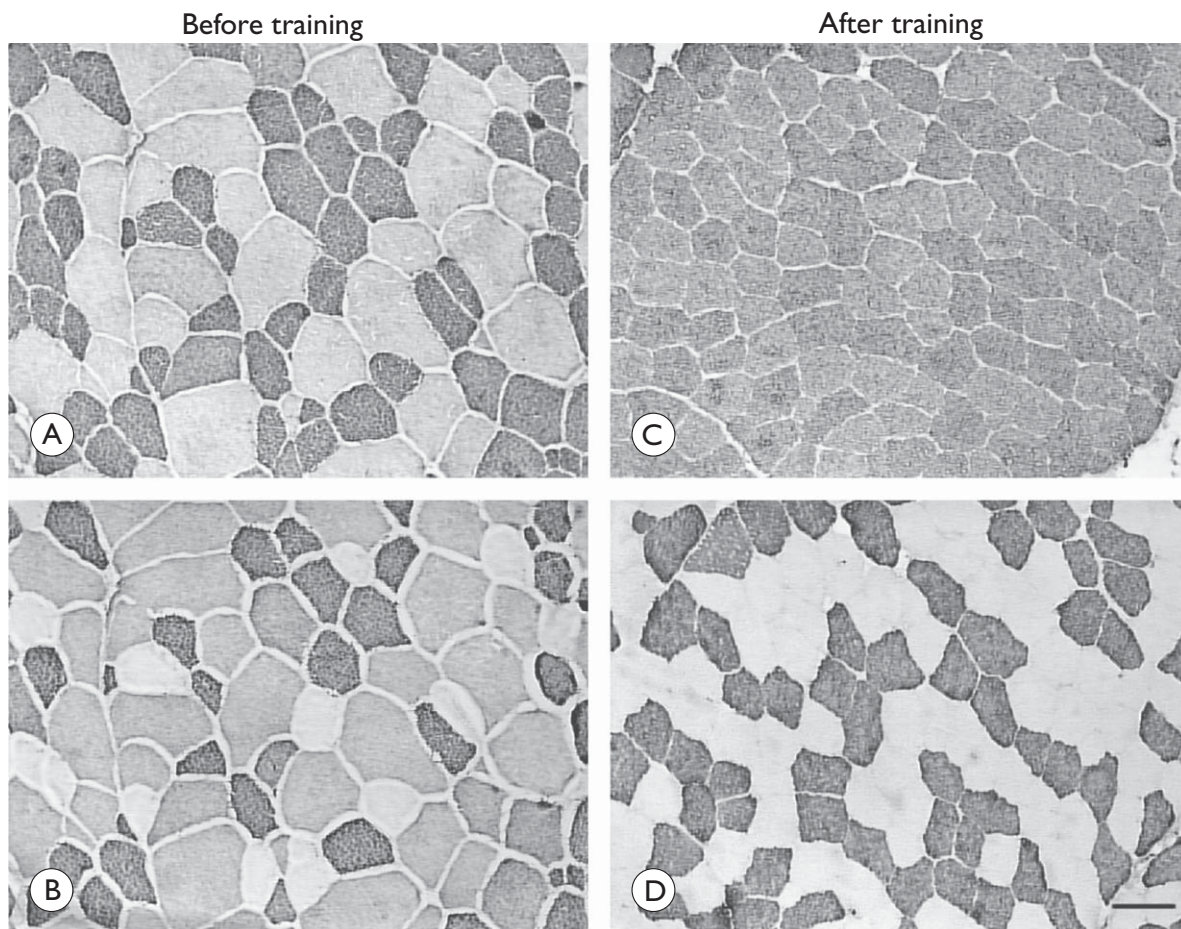


Fig. 2.1.36

Transverse serial sections of *M. gluteus medius* biopsies (depth, 6 cm) of the same horse before (A and B) and after (C and D) a long-term endurance training program (9 months in total). (A and C) Sections are stained by immunohistochemistry with a monoclonal antibody to types I or IIA myosin heavy chain isoforms; note that almost all muscle fibers express either or both of these isoforms after training. (B and D) The same sections stained by immunohistochemistry with a monoclonal antibody specific to type IIA myosin heavy chain isoform; note the significant increase in the number of fibers expressing this isoform after 9 months of training. Scale, 50 μm . Details of the training program are described in reference⁸⁴.

ing.^{5,86,104,106,204} However, high-intensity training ($\sim 80\text{--}100\%$ of $\dot{V}\text{O}_{2\text{max}}$, 5 minutes $\times$ 2, 5 days/week for 12–16 weeks) for both young¹⁷⁴ and adult²⁰⁵ Thoroughbreds did result in increased glycolytic phosphofructokinase enzyme activity. Histochemical assessment of glycerol-3-phosphate dehydrogenase enzyme activity, an enzyme well correlated with other glycolytic enzymes, was also increased by exercise consisting of alternating days of either high-intensity, short-duration ($\sim 100\text{--}140\%$ of the speed that results

in a blood lactate of 4 mmol/L (or V4) for 15 minutes) exercise or moderate-intensity, long-duration ($\sim 65\%$ of V4, 60–90 minutes) exercise for a total of 5 weeks.¹⁹³ Although training also causes increased AMP deaminase activity along with other purine nucleotide cycle enzymes, such as creatine kinase (discussed in reference⁵), the concentration of total nucleotide stores is usually not affected by training.¹⁹⁹ Nevertheless, moderate-intensity ($\sim 55\%$ of $\dot{V}\text{O}_{2\text{max}}$) and long-duration (60 minutes, over $\sim 13\text{--}14$ km) exercise for 10 con-

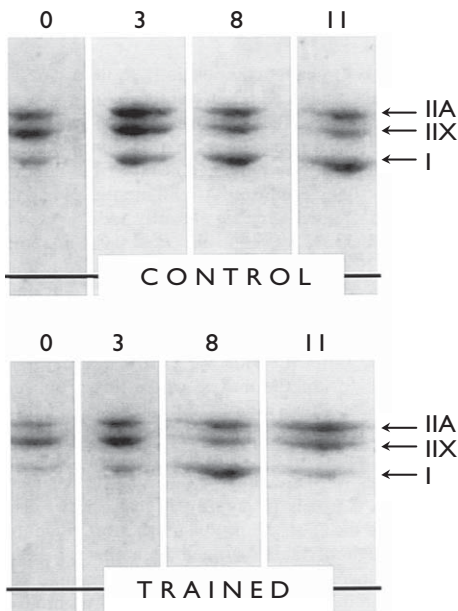


Fig. 2.1.37

Eight percent sodium dodecyl sulfate polyacrylamide gel electrophoresis of muscle samples from the gluteus medius muscle of a control horse (upper row) and a trained horse (bottom row) throughout an experiment to investigate the effects of prolonged endurance exercise training and detraining program. 0, pre-training; 3 and 8, after 3 and 8 months of training; 11, after a further 3 months of detraining. More details of the experiment in Serrano & Rivero.¹⁰³ The three myosin heavy chain isoforms are identified as I, IIA, and IIX. Note the effect of training on the relative densities of the I and IIX bands, indicating a fiber type transition in the order IIX → IIA → I.

secutive days increases muscle phosphocreatine concentration and reduces muscle creatine content at the point of fatigue.²⁰⁴

Training has also been shown to result in a modest increase in muscle glycogen storage^{104,189,193,202,206} that may well be associated with reduced activities of glycolytic enzymes, since the capacity to mobilize endogenous glycogen is partially influenced by the absolute activity of anaerobic enzymes expressed within muscle fibers.⁷⁷ In experimental animals training is known to increase the sensitivity of glucose uptake across the sarcolemma via increased GLUT-4 expression.²⁰⁷ In horses, moderate-intensity exercise training increases middle gluteal muscle GLUT-4

protein content, but this change is not reflected by increased sarcolemmal glucose transport in post-exercise muscle samples.²⁰⁸ Finally, the transfer of FFAs from the vascular to the intracellular compartment is also enhanced with endurance training, and is promoted by increased extracellular (interstitial) albumin concentration.²⁰⁹

Physiological adaptations and buffering capacity

Training has significant effects on the electrical and ionic membrane properties of equine skeletal muscle.^{210–212} Short-term exercise training of moderate intensity results in an increase in the density of Na⁺/K⁺-ATPase pumps measured by ouabain-binding assays, together with an attenuation in K⁺ efflux from working muscles during high-intensity exercise. Although the physiologic implication of these training-induced adaptations is unclear, enhanced ionic control at the sarcolemma may result in myofibers that are better able to respond to the rate of motor neuron discharge.²¹³ Additional responses following physical conditioning include increased SR calcium uptake (Ca²⁺-ATPase activity) and an attenuation of the exercise-induced decline of calcium uptake.²¹⁴

Several studies have reported an increase in buffering capacity of equine skeletal muscle after a few weeks of sprint training^{197,215–217} and after prolonged (34 weeks) training.²⁰² This increase may be due to (1) increased incorporation of myofiber protein, (2) increased creatine phosphate concentration, or (3) higher muscle carnosine concentrations,¹⁵⁴ although with regard to the latter, no differences in carnosine concentration were found in muscle from either trained or untrained Thoroughbreds.²¹⁸

Other training consequences

Training increases the expression of heat shock proteins¹⁵⁰ and splice variants of neuronal nitric oxide synthase,⁹⁶ which protect muscle cells from exercise-induced oxidative stress. Furthermore, the reported apoptosis that occurs in trained equine skeletal muscle²¹⁹ probably promotes the work/recovery/rebound/supercompensation cycle, when unconditioned muscle cells undergo programmed cell death, to be replaced by new, stronger cells. In fact, training attenuates exercise-induced ultrastructural damage in equine skeletal muscle,¹⁷⁶ probably via a parallel fiber

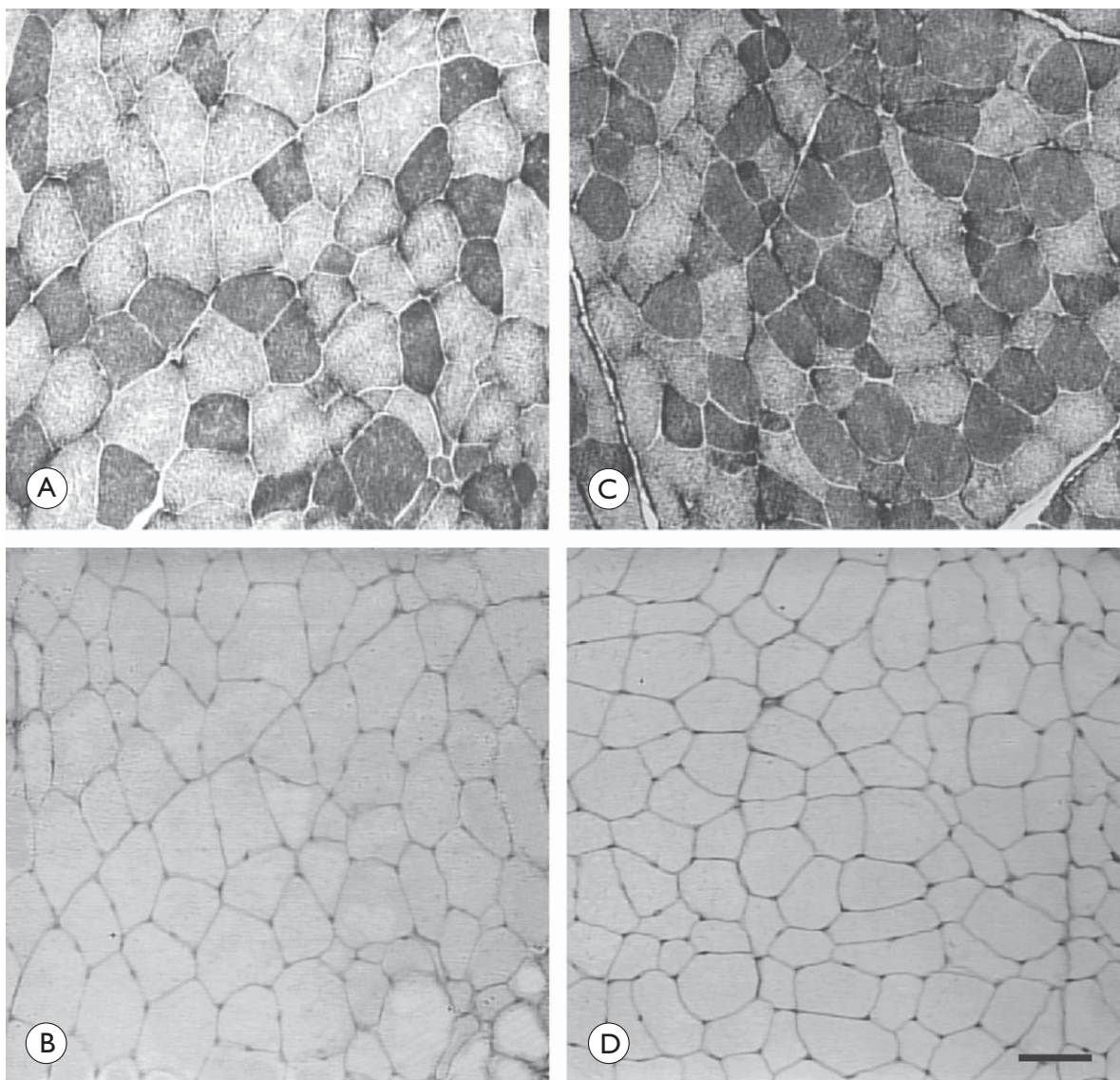


Fig. 2.1.38

Transverse serial sections of muscle biopsy samples from the M. gluteus medius from the same horse taken before (A and B) and after (C and D) 9 months of prolonged endurance training. (A and C) Sections are stained with succinate dehydrogenase to demonstrate the oxidative capacity of individual muscle fibers; note the increase in the number of fibers with dark staining after training. (B and D) Sections are stained with the α -amylase PAS to visualize capillaries; note the increased capillary density (e.g. number of capillaries per mm^2) after the training program. Scale, 75 μm .

type transition, myofiber hypertrophy or increased satellite cell activation (or perhaps all three).

Overtraining

Overtraining is a syndrome that has been recognized as a cause of poor performance in racehorses over the

course of a high-intensity and prolonged (more than 4 months) training.^{16,202,220} However, clinical signs of overtraining are accompanied by relatively few characteristic changes in skeletal muscle. In the study by Tyler and associates,¹⁶ overtraining caused significant type IIA fiber atrophy (~8%), a decrease in type IIA: IIX mATPase fiber ratio, and increased mitochondrial

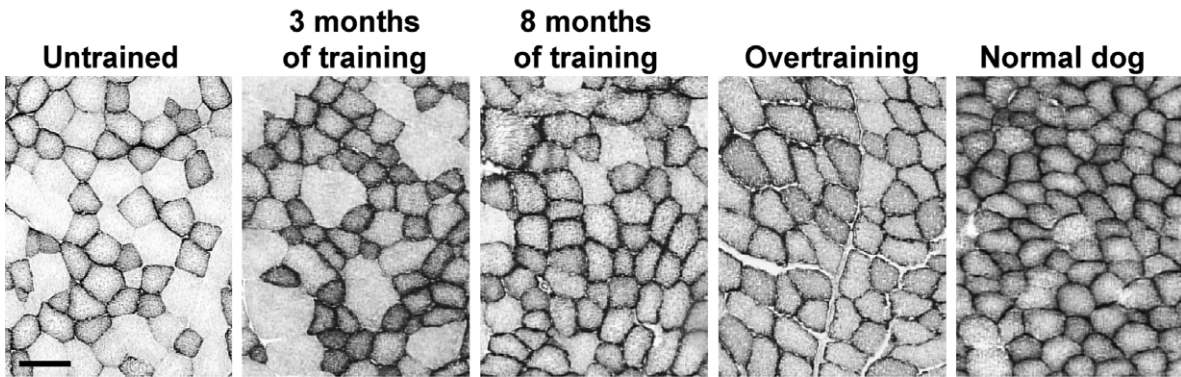


Fig. 2.1.39

Cryosections of the M. gluteus medius stained for succinate dehydrogenase activity to demonstrate the oxidative capacity of individual myofibers of an endurance horse with clinical signs of overtraining due to heavy overload and prolonged (more than 1 year) training. Microphotograph is compared with equivalent images of muscles samples from untrained and trained horses and with a sample, also from the M. gluteus medius, of an untrained dog. Note the more uniform staining pattern in muscle from the overtrained horse in comparison with the untrained and trained horse samples, a pattern that is somewhat similar to that seen in normal canine muscle. Bar scale, 50 μm .

volume in type I (~16%) and type II (~39%) fibers, accompanied by an increase in $\dot{V}\text{O}_{2\text{max}}$ (~8%). As a consequence, muscle fibers of overtrained horses are generally highly oxidative (Fig. 2.1.39).²²¹ Additional metabolic adaptation of overtrained muscle includes ATP depletion²²⁰ and reduced resting glycogen concentration,²⁰² though this latter finding may relate to delayed glycogen replenishment seen in normal horses, particularly given that glycogen utilization during exercise itself is not influenced by overtraining.²⁰²

Detraining

Maintenance of the trained muscle signature during inactivity is more prolonged in horses than other athletic species, lasting throughout 5–6 weeks of inactivity,^{16,189,191} although not beyond 12 weeks.^{102–104,222} Researchers have suggested that expression of the MyHC-IIX gene constitutes a default setting that may be altered (decreased) by chronic increased contractile activity (i.e. training), and compensated for by increased expression of MyHC-IIA.²²³ In line with this hypothesis is the observation that a return to sedentary activity levels following a prolonged endurance training period normalizes the expression of MyHC-IIX, via a slow-to-fast fiber type transformation in the order I $\rightarrow$ IIA $\rightarrow$ IIX (see Fig. 2.1.37).¹⁰⁴ These detraining-induced changes in MyHC phenotype parallel a reversion of the muscle's size and return of the muscle's metabolic and capillary characteristics to pre-training levels.¹⁰⁴ In summary, fiber sizes decrease,

together with a decline in mitochondrial density, aerobic enzyme activities and glycogen content, and normalization of anaerobic enzyme activities.

Possible mechanisms underlying muscular adaptations to training

Skeletal muscle responds to altered functional demands by specific quantitative and/or qualitative alterations in gene expression (Fig. 2.1.40), provided the stimuli are of sufficient magnitude and duration.²⁰⁰ Repeated or persistent elevation of neuromuscular activity (i.e. during exercise and training) induces a series of concerted changes in gene expression, evoking either myofiber hypertrophy or myofiber remodeling, or both.¹⁷³ Myofiber hypertrophy is generally characterized by a coordinated increase in abundance (per fiber) of most protein constituents. To some extent this process includes the selective and transient activation of specific genes immediately following the onset of work overload. The major events underlying muscle hypertrophy, however, involve a general and non-specific augmentation of protein synthesis within cells. Remodeling of myofiber phenotype, with minimal or no hypertrophy, is the typical muscular response to prolonged training in the horse.¹⁰⁴ During this type of adaptation, myofibers undergo striking reorganization, with selective activation and repression of many genes, and switching between different myofibrillar isoproteins occurring in a graded and orderly sequen-

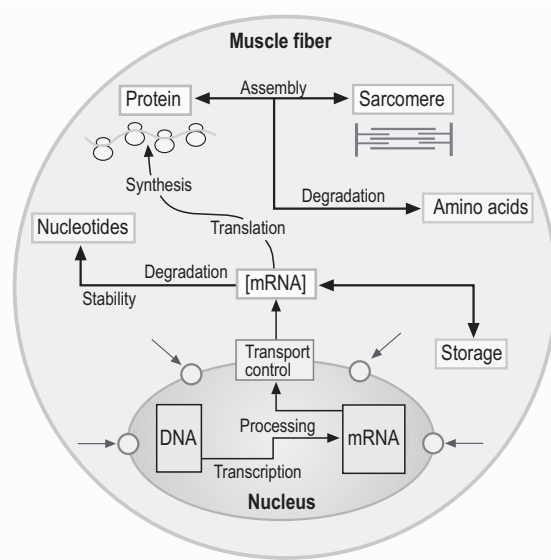


Fig. 2.1.40
Schematic diagram showing different steps in the regulation of gene expression in skeletal muscle associated with increased contractile activity.

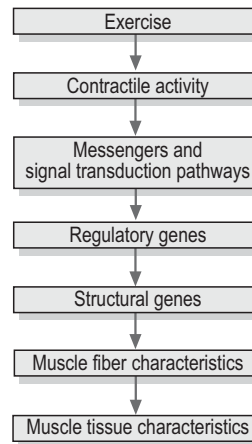


Fig. 2.1.41
Steps in the cascade of events by which exercise and increased neuromuscular activity lead to physiologically relevant changes in the characteristics of skeletal muscle.

tial manner.²⁰⁰ These changes occur in parallel, but not simultaneously over time, and correspond to the changes observed in enzymatic profiles, cytosolic proteins, and membrane receptors and transporters.

The complexity and pleiotropic nature of the physical and metabolic stimuli presented to myofibers during contraction that ultimately results in altered gene regulation were reviewed by Williams & Neuffer.¹⁷³ Acetylcholine released from motor neurons and other signaling molecules of neural origin bind to cell surface receptors on myofibers, triggering intracellular events that may be linked to altered gene expression (Fig. 2.1.41). Additional signals are probably derived from contraction-induced mechanical stress perturbing the sarcolemma and extracellular matrix, thus exerting tension via intermediate filaments on the cytoskeleton, organelles, and nucleus.²²⁴ Changes in the intracellular concentrations of ions and metabolites during repeated muscle contraction are also implicated in the activation of signaling pathways.⁷⁷ These signals include intracellular calcium, hydrogen ions produced during anaerobic exercise, the marked reduction in phosphorylation potential of the adenylate system ($[ATP]/[ADP_{free}]$), a depletion of the redox state ($NADH/NAD$), and hypoxia. Among all these factors, an imbalance between energy requirement and energy supply is

possibly the most important signal that triggers appropriate adjustment of contractile and metabolic protein expression.²²⁵ Finally, recent years have seen significant advances in our understanding of the signaling mechanism by which the information contained in specific action potential patterns alters transcription within muscle fiber nuclei.²²⁶ For example, Ras-mitogen-activated protein kinase²²⁷ and calcineurin²²⁸ signaling are implicated in the α -motor neuron induction of slow muscle fiber phenotype, but not muscle growth. Conversely, a protein kinase B-dependent and rapamycin-sensitive pathway controls myofiber growth but not fiber type specification.²²⁹

Specific genes known to undergo altered expression following contractile activity include those encoding sarcomeric and cytosolic proteins and enzymes of the glycolytic pathway, TCA cycle, electron transport chain, and fat oxidation.¹⁷³ Additionally, signals inducing the expression of proteins derived from mitochondrial genes are coordinated with the activation of the various nuclear genes that encode mitochondrial proteins.

The factors that promote angiogenesis in skeletal muscle in response to training have not been clarified, although they may be related to a chronic increase in muscle capillary blood flow and the corresponding endothelial shear stress, as well as increased capillary wall tension.²³⁰ Hudlicka and colleagues speculated that endothelial stress may disturb the luminal surface, resulting in the release of bound proteases that damage

the basement membrane and contribute to an increase in basic fibroblast growth factor release.²³⁰ Subsequently, growth factors may enhance vascular growth and satellite cell proliferation.²³¹ Still unclear, however, is the influence of training intensity and duration on neovascularization and the mechanisms that underlie the increase seen in intramuscular substrates in response to long-term endurance training. These latter adaptations may be related to either (1) increased glucose and FFA availability (via GLUT-4 and albumin respectively), (2) a lower utilization of these substrates for energy production, or (3) possible artifacts imposed by experimental design (i.e. they may be a reflection of increased dietary intake of soluble starches and fat from a parallel change in diet in horses in training).⁵

Implications of training-induced changes to the physiologic response to exercise

The main physiologic consequence of increased muscle mass in response to training is to produce a muscle with a greater peak force capacity, because force output is proportional to total cross-sectional area of the fiber mass recruited.²¹³ At slow speeds, this adaptation has an impact on gait, causing a marked reduction of both stance time and stride duration.¹¹⁵ This adaptation significantly affects the performance of showjumpers via enhanced power output from the hindquarters.¹⁸⁴ Furthermore, because increased power output results in a greater ability to accelerate and may increase stride length, these training adaptations (strength rather than endurance) may be important for race horses competing over short distances.⁵ However, enhanced power through training comes with the cost of a corresponding decline in aerobic potential, because the increased mass of recruited fibers and concomitant rise in ATP utilization occur simultaneously with a relative inability of oxygen to diffuse into the larger fibers.¹⁹¹

From a physiologic standpoint, remodeling of myofiber phenotype with minimal or with no hypertrophy in response to training, produces a muscle that is much more resistant to fatigue but with decreased maximal shortening velocity, the former corresponding to each myofiber's increased oxidative capacity, and the latter to the increased expression of slow MyHC and other contractile protein isoforms.¹⁸ In a similar but reciprocal fashion to that described for strength training, some conventional training programs of young race horses produce a decrease in

the size of type II fibers¹⁹² and a corresponding decline of both speed and force of contraction.⁷⁶ Clearly a balance must be acquired at a level deemed most appropriate for the intended use of the horse (see below).

Following endurance training, exercise at submaximal intensity is facilitated by optimal delivery of oxygen and blood-borne substrates, early activation of oxidative metabolism with low utilization of endogenous carbohydrates, and increased reliance on fat oxidation as an energy source. The increased oxidative capacity observed in skeletal muscle after training occurs concurrently with increased maximum oxygen uptake¹⁶ and a significant reduction in the net rate of muscle glycogenolysis and anaerobic metabolism.²⁰⁴ As a consequence, in the trained state the speed at which a horse begins to accumulate lactate increases gradually (i.e. there is a delay in the onset of lactate accumulation and ATP depletion).^{76,180,181} This is accompanied by enhanced muscle buffering capacity and more efficient excitation–contraction coupling. Hence, collectively, endurance may be enhanced by a wide variety of related factors that delay the onset of fatigue during anaerobic exercise and, although relative sparing of muscle glycogen underlies the delay in fatigue onset during this type of exercise in the trained state, it is likely that all the metabolic adaptations summarized above combine to increase endurance.

Although controversial, then, it is clear from extensive scientific literature that equine skeletal muscle has considerable potential to adapt during training, and overall, that these adaptations have important functional implications affecting stamina, strength and speed (Table 2.1.2). Ideally, therefore, conditioning programs of athletic horses should promote characteristics that optimize equilibrium between these physiological traits.⁵ While interpreting Table 2.1.2, first note that certain changes occur together, whereas others occur independently. For example, reduced intrinsic muscle shortening velocity occurs together with increased endurance capacity following IIX → IIA fiber transition, whereas increased aerobic muscle enzyme activities are never associated with decreased mitochondrial volume. Second, note that certain changes precede others; for instance, aerobic metabolic adaptation occurs prior to any structural adaptation. Thus significant increases in muscle glycogen concentration occur after only 10 consecutive days of training,²⁰⁴ whilst a significant degree of type II → type I fiber type conversion needs much longer (8 months).¹⁰⁴ Third, adaptations are cumulative and

Table 2.1.2 Summary of main muscular adaptations to training reported in horses and their functional significance for endurance, strength, and speed

	Physiological trait			
Muscular adaptations	Stamina	Strength	Speed	References
Morphological adaptations				
Muscle fiber hypertrophy	–	+ + +	+	16,86,102–104,114,175,177,183–187,
Muscle fiber atrophy	+	– – –	–	179, 190–193
Increased number of capillaries	+ + +	nc	nc	16,102,113,197,203
Increased mitochondrial volume	+ + +	nc	nc	16
Increased myonuclear density	nc	+ + +	nc	Not reported in horses
Metabolic adaptations				
Increased aerobic muscle enzyme activities	+ + +	nc	nc	86,104,106,120,175–177,184,199,201,202
Increased glucose and fatty acid transport	+ + +	nc	nc	207,208,209
Increased muscle [glycogen] and its sparing during exercise	+ + +	nc	nc	104,189,193,202,206
Increased muscle [triglycerides]	+ + +	nc	nc	104
Decreased post-exercise muscle [lactate]	+ + +	nc	nc	76,180,181
Increased anaerobic muscle enzymes	nc	nc	+ + +	175,193,205
Decreased anaerobic muscle enzymes	nc	nc	– – –	5,86,104,106,204
Increased muscle [high energy phosphate]	nc	nc	+ + +	204
Increased muscle buffering capacity	+	nc	+ + +	76,180,181
Contractile adaptations				
Unidirectional IIX→IIA→I fiber type (MyHC isoform) transition	+	nc	– – –	16,86,96,102–104
Bidirectional IIX→IIA→I fiber type (MyHC isoform) transition	+	nc	– – –	193
Increase of IIA:IIX fiber type (MyHC isoform) ratio	+	nc	– – –	190,191,195–197
Increase of I:IIA fiber type (MyHC isoform) ratio	+	nc	– – –	103
Increase of IIA:I fiber type (MyHC isoform) ratio	–	nc	+ + +	193

+++ and --- = primary implication (positive and negative, respectively) towards the particular characteristic; + and – = secondary implication (positive and negative, respectively); nc = no contribution to the particular characteristic.

dose-dependent, but there is an upper limit above which further adaptations do not occur, even when the stimulus continues.¹⁶ Most relevant training adaptations, then, occur in the first 3–4 months; prolonging training beyond this period, although improving aerobic capacity, reduces anaerobic capacity and has no effect on strength. Simultaneously, the risk of overtraining increases. Finally, certain adaptations to training are reversible, tending to return to the pre-training situation when the stimulus is stopped.

The response of equine muscle to training therefore depends on two sets of factors: (1) the basal status of the muscle (determined by the breed, age, sex, and level of fitness of the horse), and (2) the stimulus applied (i.e. type, intensity, duration, frequency, and volume of the training exercise). Unfortunately, little is known about the relative influence of most of these factors. For example, only a few studies compare muscular adaptations that occur with different training programs. Of these, two studies (with contradictory results) examine the influence of different exercise

Table 2.1.3 Physiological implications of muscular adaptations in various training programs scientifically evaluated in horses

Horses		Conditioning protocol				Functional significance			
Breed	Age	Intensity	Duration (Distance)	Frequency	Volume	Stamina	Strength	Speed	Reference
Thoroughbred	4-8 yr	~55% $\dot{V}O_{2\text{ max}}$	60 min (~13-14 km)	Daily	10 days	+	ni	-	204
Thoroughbred	2-9 yr	~80% $\dot{V}O_{2\text{ max}}$	3 min (1500 m) $\times$ 2	6 d/wk	6 wk	+	ni	-	197
Thoroughbred	5-7 yr	~80-100% $\dot{V}O_{2\text{ max}}$	5 min ($\times$ 2)	5 d/wk	12 wk	ne	ni	++	205
Thoroughbred	2-3 yr	~100-165% $\dot{V}O_{2\text{ max}}$	1.6-5.3 min (1600-3200 m)	5 d/wk	16 wk	++	+	-	86
Thoroughbred	2 yr	~100-165% $\dot{V}O_{2\text{ max}}$	1.6-5.3 min (1600-3200 m)	5 d/wk	16 wk	++	+	+	175
Standardbred	3-5 yr	~60-100% $\dot{V}O_{2\text{ max}}$	6-12.5 min (~3-9 km)	5 d/wk	16 wk	++	++	ne	16
					32 wk	++	+	-	
Standardbred	2 yr	~100-140% V4	15 min	2nd day	5 wk	+	-	+	193
		~65% V4	60-90 min	2nd day					
Arab	8.6 yr	~80% V4	50-80 min (~10-20 km)	3 d/wk	12 wk	+	+++	-	187
Andalusian	~4 yr	~25-30% V4	45-60 min	5 d/wk	12 wk	+	+	-	104
		~50-60% V4	75-120 min		32 wk	+++	+	-	

Intensity is expressed as a fraction of either $\dot{V}O_{2\text{ max}}$ (velocity at maximal aerobic capacity) or V4 (velocity inducing a blood lactate concentration of 4 mmol/L). The symbols + and – indicate that either the muscular adaptations to training had positive or negative effect respectively towards the particular characteristic; the number of symbols is proportional to the magnitude of the adaptation; ne = no effect; ni = not investigated.

intensity,^{197,201} and only one study has examined the combined influence of intensity and duration.²⁰⁶ This latter study concluded that low-intensity exercise (~50% of V4) for long duration (45 minutes) was, after 6 weeks, more effective in improving aerobic capacity exercise than high-intensity exercise (~100% of V4) of moderate duration (25 minutes). Recent years have, however, seen the publication of several experimental studies with well-documented exercise protocols (Table 2.1.3). From these it seems that moderate- to high-intensity (~80–100% of $\dot{V}O_{2\text{ max}}$) exercise of short duration (5–10 minutes) improves both stamina and strength in race horses following 12–16 weeks of training,^{16,86} whereas anaerobic capacity can only be increased in the short to mid-term (up to 16 weeks), by introducing supramaximal intensity exercise (~100–150% of either $\dot{V}O_{2\text{ max}}$ or V4) of short (2 minutes) to moderate (15 minutes) duration.^{175,193,205}

Interestingly, adaptation occurs more readily in younger (~2-year-old) than in mature race horses; for instance, muscular adaptations compatible with a combination of improved stamina, strength, and speed were reported in young Thoroughbreds following exercise of high (~100% of $\dot{V}O_{2\text{ max}}$) to very high (~165% of $\dot{V}O_{2\text{ max}}$) intensity and short duration or distance (1.6–5.3 minutes; 1600–3600 meters) 5 days/week for 16 weeks.¹⁷⁵

In general, though, it seems clear that improved stamina through enhanced aerobic capacity is the most common response of equine skeletal muscle to training, regardless of either the basal status of the muscle or the training–exercise program. Given that many equestrian disciplines, including Thoroughbred racing, are largely aerobic in nature, this underlies the likely benefit of training to the equine athlete.

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Skeletal physiology: responses to exercise and training

Allen E. Goodship & Roger K.W. Smith

Introduction

Role of the skeleton

The mammalian skeleton provides protection for vital organs, a reservoir of minerals, and a structural support for the body. Its major role, however, is provision of structural support and a means of locomotion using jointed bones together with muscles, tendons, and ligaments. These components have evolved to optimize posture and locomotion for the lifestyle of individual species. The type of joint and position of muscle and tendon attachment relative to lever arms and fulcrum provide an optimal mechanical advantage. As a structure the skeleton has evolved to provide maximum strength with minimal mass. The skeleton comprises a series of morphologically distinct elements — bones. The shape and size of these individual bones are determined by genetic and functional factors to provide an appropriate structure for functional demands with low risk of failure and without incurring excess energy expenditure. The demands for energetic efficiency are greater in animals evolved for high-speed locomotion, such as horses.

The horse has not only evolved to become a high-speed animal, but has also been the subject of selective breeding as an elite animal athlete. The Thoroughbred race horse is a prime example of selection for speed. However, it has been suggested that this selection process has reached its limit on the basis of classic race times remaining similar for many years. The Thoroughbred has arisen from a somewhat limited gene pool and this may have resulted in a plateau of performance.¹ This is in contrast to the performance of human athletes in which records are broken almost year on year. However, an alternative explanation is that training methods to condition race horses have not developed to optimize the capacity of the adaptive

responses of the musculoskeletal system. In human athletics the application of sports science has undoubtedly contributed to the enhancement of performances over recent years. Equine sports science is not yet developed or applied to the same extent. In addition, many training systems continue to be based on empirical and traditional methods.

The skeleton has a unique capacity to respond to changes in mechanical loading in the short term and can, therefore, optimize for energetic efficiency in relation to changes in mechanical demands. There is a need not only to understand these mechanisms in general but also to apply the information to specific training regimens for equine athletes.

Skeletal requirements for the horse

The requirements for the skeleton in the horse are, in common with other animals, related to functional demands, imposed by both evolutionary process and short-term conditioning. Domestication and the varied requirements for man's interaction with this species have resulted in a wide range of breeds and types of horse, with great variation in conformation. Selective breeding has resulted in adaptation of this species for the different specific purposes. Some breeds are massive and the bones are relatively high in mass to accommodate needs for strength and endurance but they are not able to sustain high speeds. Others have been selected for speed, the Thoroughbred race horse perhaps being the best example of skeletal development for speed. As such, the requirements for the skeleton in these horses are those of low mass and high strength.

The 'design' of the equine skeleton is a refinement of the basic mammalian pattern. The horse is an

unguligrade animal, a member of the order Perissodactyla, the odd-toed ungulates standing on the hoof of the third digit. The major muscle masses of the limbs are positioned proximally to reduce the energy required to move the limb as it swings backward and forward in cursorial locomotion. Muscle forces are transferred to distal bones across joints by means of long tendons. The tendons and ligaments have also evolved to play a major role in the energetic efficiency of locomotion, many acting as energy-storing springs.

Bone mass is also minimized at the distal extremities and safety margins decrease toward the distal extremity of the limbs. This has been related by Currey² to the incidence of fractures in race horses³ being higher in the distal bones than those located proximally.

Selection for high-speed gait is also reflected in the morphology of the equine skeleton. The lever arms at the elbow and hock result in small muscle contractions producing fast extensive movement of the lower limb segments.

Skeletal refinements in the elite equine athlete are basically fine-tuning of this general pattern. New methods of analysis of skeletal conformation are being used to assess the level of performance in several different types of horse.⁴ However, these systems have not yet been perfected any more than many other methods for identifying potential winners at an early age. With advances in understanding of the factors that optimize efficiency and the development of complex computer modeling systems, it may eventually be possible to identify athletic performance of individual horses at an early age.

The ability to select and train elite equine athletes must result from a sound understanding of many aspects of the pathobiology of the horse, from the cell and molecular level to that of the whole animal. One of the most important systems in relation to locomotor performance is the musculoskeletal system. The major structural support tissue is bone.

Bone as a tissue

Bone as a tissue is a member of the family of connective tissues. These are characterized by being a composite of cells and extracellular matrix. The matrix in the case of bone is a composite of an organic, predominantly collagenous component and an inorganic hydroxyapatite component.

Bone cells and interactions

Adult bone tissue comprises three major populations of cells, each with a specific functional role but with both cell-to-cell interactions and also cell-matrix interactions. It is the coordinated interaction of the activities of these cell populations that optimizes the morphology of bones in relation to changing mechanical demands. Understanding these mechanisms and applying the principles to training regimens has the potential to improve performance and minimize injury in conditioning of equine athletes.

Osteoblasts

These cells are derived from local lining cells. The flattened mononuclear cells become plump when activated and synthesize bone matrix in the form of osteoid (Fig. 2.2.1). The osteoid then becomes mineralized over a period of weeks to form bone matrix. In rapidly forming surfaces some osteoblasts are entrapped in their own matrix and these then become osteocytes (Fig. 2.2.2). The osteoblasts communicate with osteoclasts and enable activation of osteoclasts to allow bone resorption. Osteoblasts also produce colony-stimulating factor that increases numbers of pre-osteoclasts from mononuclear precursors in the bone marrow, and also osteoclast activation factor that activates the pre-osteoclasts and initiates resorption of bone matrix (Fig. 2.2.3). The coupling of bone resorption and subsequent bone formation has recently been

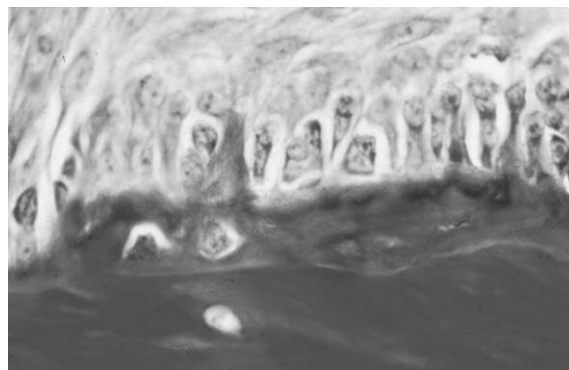
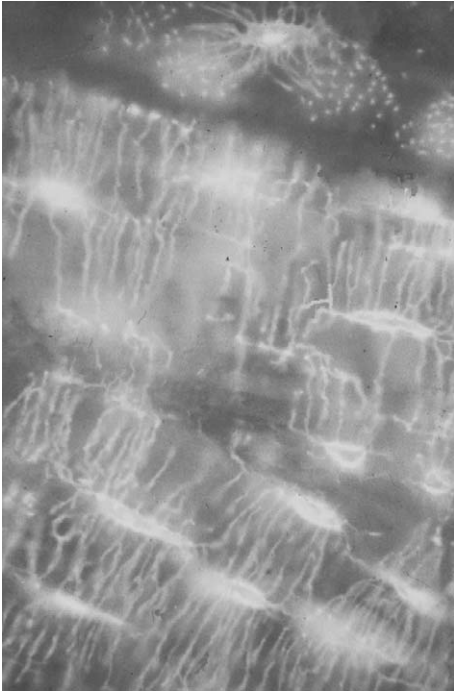
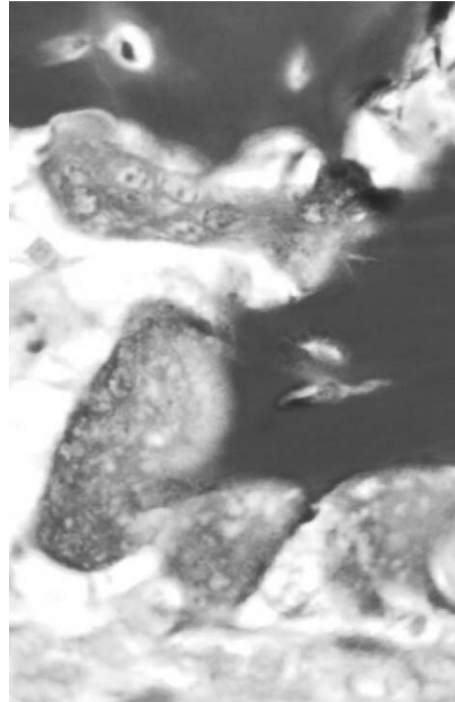


Fig. 2.2.1

A photomicrograph of bone showing the bone-forming cells called 'osteoblasts'. The dark staining matrix is the pre-calcified osteoid. Cells can be seen becoming entrapped in matrix to become osteocytes.

**Fig. 2.2.2**

Bone cells — osteocytes entrapped within bone matrix showing communication via cytoplasmic processes within canaliculi.

**Fig. 2.2.3**

A photomicrograph showing bone resorption by osteoclasts.

shown to involve a receptor on the osteoblast cell membrane known as RANK ligand (RANKL), which binds to RANK present on the surface of pre-osteoclasts and induces activation of the intracellular cascades to activate the osteoclasts. The RANKL can also bind to a protein called 'osteoprotegerin,' OPG, which prevents binding with and activation of osteoclasts. Thus a regulation of coupling of these cells and associated amounts of bone resorption and formation is effected by this system (Fig. 2.2.4).^{5,6} Other systemic factors can also influence this system; for example, parathyroid hormone (PTH) can 'blunt' OPG influence. The osteoblasts can also secrete collagenase, an enzyme that removes the surface layer of osteoid, unmineralized bone matrix, on bone surfaces and allows osteoclasts access to the bone matrix. Boyde et al used in vitro systems to measure bone resorption activity of osteoclasts and also to show the interactions between osteoblasts and osteoclasts.⁷ Hormonal influences on calcium metabolism and bone resorption act indirectly on receptors on the osteoblast, which in turn regulates osteoclast recruitment and activity. Thus the osteoblast is central to

the control of the bone modeling and remodeling process.

Osteoclasts

These cells are derived from circulating monocytes. They are multinucleate cells (see Fig. 2.2.3) which, when activated, reside on a bone surface with a ruffled border to isolate the local environment between the cell and the bone surface. The perimeter of the cell membrane forms a seal against the underlying bone involving adhesion molecules, the integrins, to isolate the local environment beneath the cell, which is then lowered in pH by an active proton pump generating hydrogen ions.⁸ The pH falls to around 2–3 and the bone matrix and embedded osteocytes are resorbed, forming a resorption pit, and the resorption products are trafficked through the cell.⁹ As indicated above, this process is regulated by the osteoblasts, which in turn communicate with the third population of cells — the osteocytes. The bone-resorbing osteoclasts are also influenced directly by some specific hormones such as calcitonin.

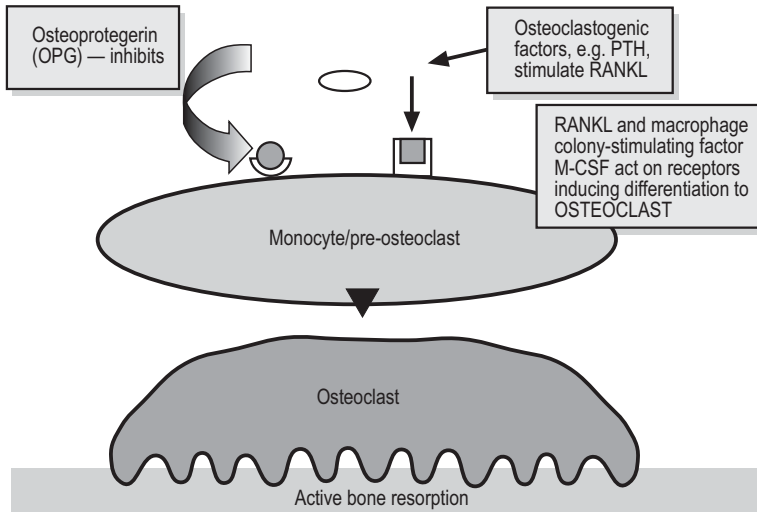


Fig. 2.2.4
Coupling of the bone remodeling cycle.

Osteocytes

These cells arise from osteoblasts that become trapped in the bone matrix within lacunae. They are cells with many long cytoplasmic processes within small tunnels in the matrix called canaliculi (see Fig. 2.2.2). Processes from adjacent cells connect by means of gap junctions allowing cell-to-cell communication. A finding by Skerry and co-workers has been the identification of glutamate transport systems involved in osteocyte cell signaling.¹⁰ This transmitter also operates in the central nervous system, where complex interneuronal signaling occurs. The presence of such a signaling mechanism among osteocyte bone cells provides supporting evidence that this population of cells plays a role in the overall perception of mechanical environment on a bone and coordinates an appropriate response to ensure optimal bone size and architecture.

The osteocytes and their cell processes are surrounded by extracellular fluid. The mechanical loading of a bone results in deformation and movement of this extracellular fluid within the matrix around the cells. Extracellular fluid contains ions and the movement of this ionic fluid with respect to the charged surfaces of the matrix induces electrical potentials. These electrical charges are referred to as 'streaming potentials', which are also thought to influence the cell activity and provide a putative mechanotransduction pathway.^{11,12} The gap junctions linking cell communication are also modulated

in number by mechanical loading of bone and thus may play a role in regulation of bone form in response to functional demands.¹³

The networks of osteocytes also communicate with the surface lining cells and osteoblasts. Thus the integration of the cell populations by the osteoblasts provides a complex but extremely sensitive mechanism to enable bone mass and architecture to be optimized for the changing mechanical demands throughout life.

Lining cells

Quiescent bone surfaces are covered by lining cells, which have the capacity to respond to both mechanical and biological signals and are activated to change shape into plump, metabolically active osteoblasts. These cells are found in the osteogenic layer of the periosteum or endosteum; these membranes comprise a deep cellular layer and a more superficial fibrous layer (Fig. 2.2.5). Some pluripotent cells have been demonstrated in the osteogenic cellular layer and these have been shown to have the capacity to differentiate into other connective tissues such as cartilage. There is currently considerable interest in the various types of pluripotent cell in the adult as a source of 'stem' cells that play a role in tissue regeneration. These quiescent lining cells are activated by mechanical or hormonal stimuli to generate a bone-forming front of metabolically functional osteoblasts involved in a modeling or remodeling process to maintain or restructure the matrix.

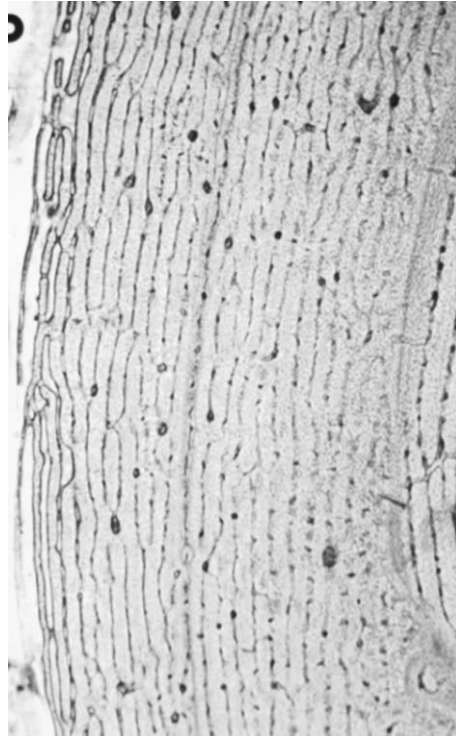
**Fig. 2.2.5**

Lining cells on a bone surface — periosteum, comprising a fibrous outer layer and an osteogenic or cambium inner layer of cells.

Bone matrix

The extracellular matrix of bone is composed of two major components: first, the collagenous organic component of predominantly type I collagen, imparting high tensile strength, and second, the inorganic salt hydroxyapatite, imparting high compressive strength. The apatite crystals are deposited onto the collagen and gradually become orientated in a preferred direction with age.¹⁴

As a composite material, bone matrix is anisotropic and the architecture of the matrix in relation to the osteocytes forms the basic unit of structure, termed the lamella. The architecture of the lamellae can be observed under polarized light microscopy as the collagen is a birefringent material. The architecture of these bone lamellae in different geometrical arrangements forms the basis of the different histological types of bone. Concentric circumferential lamellae form osteons and when these are formed in the original development of the bone they are termed 'primary osteons'. A similar architecture is also seen in the secondary osteons that are formed to repair damage such as microcracks or infill porosities within the cortex. Lamellae formed around vascular networks or plexi result in laminar or the less regular plexiform bone often seen in ungulates like the horse (Fig. 2.2.6). This type of bone allows very rapid increases in cross-sectional area with later consolidation.² Lamellar bone can also form circumferential lamella on the entire

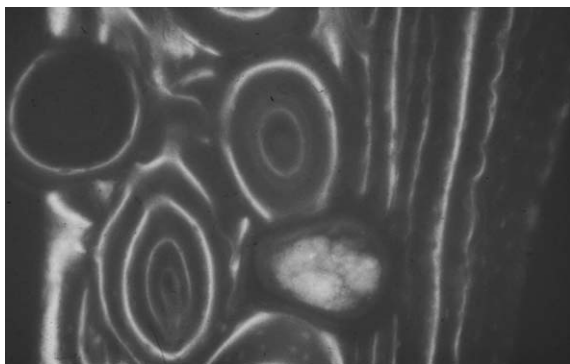
**Fig. 2.2.6**

Photomicrograph of a transverse section of laminar bone.

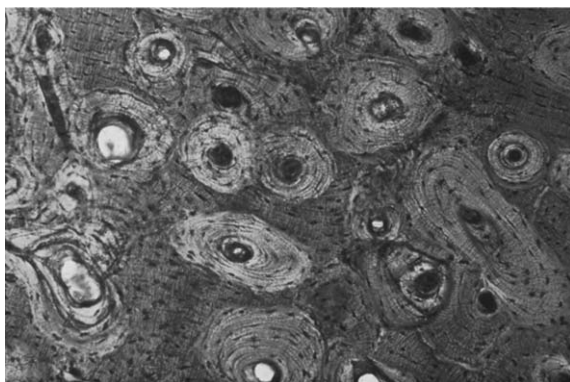
periosteal and endosteal surfaces of individual bones (Fig. 2.2.7).

In embryological development and in the early stages of fracture repair a rapidly forming bone with irregular lamellae, coarse collagen fibers, and large osteocyte lacunae is seen. This is called 'woven' bone and is rapidly remodeled to the various types of organized lamellar bone, such as primary and secondary osteonal bone, previously termed 'Haversian' bone (Fig. 2.2.8).

Bone morphology has been related to the mechanical loading requirements of different specific bones. For example, the orientation of collagen fibers in the cranial and caudal cortices of the radius reflects the mechanical requirements of this bone. Strain gauge studies have shown that this bone is loaded in both compression and bending with principal tensile strains aligned with the long axis of the bone in the cranial cortex and principal compressive strains aligned to the long axis in the caudal cortex.¹⁵ The arrangement of collagen fibers in relation to this pattern of loading has been demonstrated by Riggs et al.¹⁶ This has been

**Fig. 2.2.7**

Photomicrograph of a transverse section of cortical bone to show secondary osteons at different stages of formation in the process of remodeling and endosteal circumferential lamellar bone.

**Fig. 2.2.8**

Photomicrograph of a transverse section of highly remodeled secondary osteonal cortical bone.

supported by a comprehensive analysis of matrix morphology in the equine radius by Mason et al, in which the analysis of primary bone and bone within secondary osteons of the cranial and caudal cortices of the radius were arranged appropriately to optimize for the pattern of functional loading.¹⁷ Mason et al also confirmed a predominant longitudinal arrangement of collagen fibers in the cranial cortex of this bone, to resist the functional tensile strains at this location.¹⁷ Any consistent changes in the magnitude and pattern of loading induce a modeling response in which the bone cell activity will modify the matrix to maintain the optimization of the overall bone architecture in relation to the new prevailing loading conditions. Matrix, and embedded osteocytes, can be removed by

osteoclasts and new matrix formed by osteoblasts. This coupled cellular activity allows bone as both a material and structure to be changed in terms of mass and distribution throughout life.

Matrix molecular composition

Bone matrix comprises approximately 65% inorganic mineral, largely hydroxyapatite, and 35% organic material and water.

As with all connective tissue matrices, there is a high proportion of water in the order of 25%, dependent upon type of bone. The remaining matrix is predominantly type I collagen together with a small proportion of minor collagens and non-collagenous proteins, including proteoglycans and glycoproteins. Some growth factors such as transforming growth factor (TGF)- β and bone morphogenetic proteins are also present in abundance within bone matrix; these peptides act as biological signaling molecules for mitogenesis and differentiation of bone-forming cells. They are released with osteoclastic resorption of the matrix and can act in autocrine and paracrine manner on the osteoblasts.

Bone matrix components include osteonectin, bone sialoprotein, osteopontin, and osteocalcin, a protein that can be used in blood assays together with collagen propeptides that have been investigated as a non-invasive method to monitor bone cell activity during growth, development, and training of horses.^{18,19}

Material properties

The material properties of bone matrix vary and are largely related to the degree of mineralization, particularly with respect to modulus of elasticity. Bone such as the tympanic bulla, with a very high mineral volume fraction and density, has a high modulus eminently suitable for conduction of sound, whereas deer antler is low in mineral content and has a low modulus, again appropriate to avoid fracture in its role in fighting. Bone matrix is an anisotropic brittle elastic material and the elastic modulus is related to the level of mineralization. The influence of mineralization on material properties has also been evaluated during growth and maturation. In immature horses the level of mineralization is low and increases to a higher level with maturation to adult equine bone. With development and increased mineralization there is a significant correlation with changes in mechanical properties; it is suggested that such changes may be measured non-invasively using ultrasound

transmission systems. This would provide the potential to monitor material properties of bone during growth and possibly during training.²⁰

The material properties and morphology of specific bones determine the structural properties of the individual skeletal elements. Thus changes in the mechanical competence of bones as structures may be a consequence of either changes in the material properties of the constituent tissue or an alteration in the structural distribution of the material in relation to the magnitude and distribution of the loads applied at a particular time. The dynamic nature of bone tissue provides the ability to optimize structure in response to changes in matrix material properties.

Mechanical characteristics

Bone tissue as a material contributes to the mechanical characteristics of the individual skeletal elements. The architectural arrangement of the material is predominantly related to the general functional requirements of the particular skeletal elements. In the long bones the diaphysis is tubular to resist the functional loading patterns of bending and torsion. In engineering terms these loading patterns can be sustained with maximum strength and minimal material by tubular structures with the material distributed at a distance from the neutral axis. For example, if a solid beam is subjected to bending, one surface will experience tensile stresses and the opposite surface compression stresses. At some plane between these two surfaces there will be neither tensile nor compressive stresses (the neutral axis); thus no material is required in this region. When bone tissue organizes into the components of the skeleton, its structure is based on optimization of energetic efficiency. Thus to minimize the mass of material, bone is not present in the medullary cavity (Fig. 2.2.9). The relative thickness of the cortices also reflects the loading patterns of a particular bone. These changes may be measured using radiographic and ultrasound transmission techniques.²¹

At the extremities of the long bones and in short bones the functional loads are predominantly those of axial compression. This is reflected in a different architecture with a thin cortical shell supported by internal cancellous bone (Fig. 2.2.10). The plates and rods of bone are termed trabecula and formed of aligned bone lamellae (Fig. 2.2.11). These rods and plates are not randomly arranged but strategically placed in relation to the trajectories of principal compressive and tensile

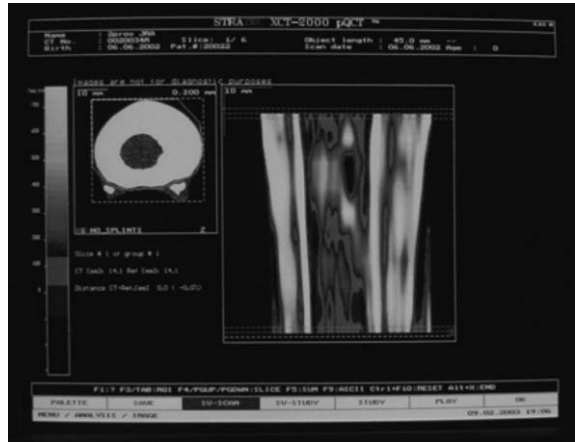


Fig. 2.2.9

Data from a pqCT scan of an equine MC3 bone to show the variation in cortical thickness and the presence of the medullary cavity as the region of the 'neutral axis'.



Fig. 2.2.10

Photograph of the metaphysis and epiphysis of a long bone sectioned longitudinally to show the cortical shell supported by internal cancellous spongy bone.

stresses. In the cancellous bone of the epiphyses, underlying the articular surfaces of the synovial joints, the trabecula are orthogonal (perpendicular) to the articular surface. This architecture of the plates and rods again allows the use of minimal material to provide maximum strength and minimize energy requirements for locomotion. In short bones that are

loaded predominantly in compression, the internal structure is of strategically arranged bony trabecula. Some short bones, such as the calcaneus, are subjected to bending moments. The internal cancellous architecture reflects this with arcades of trabecular bone arranged orthogonally to resist the principal

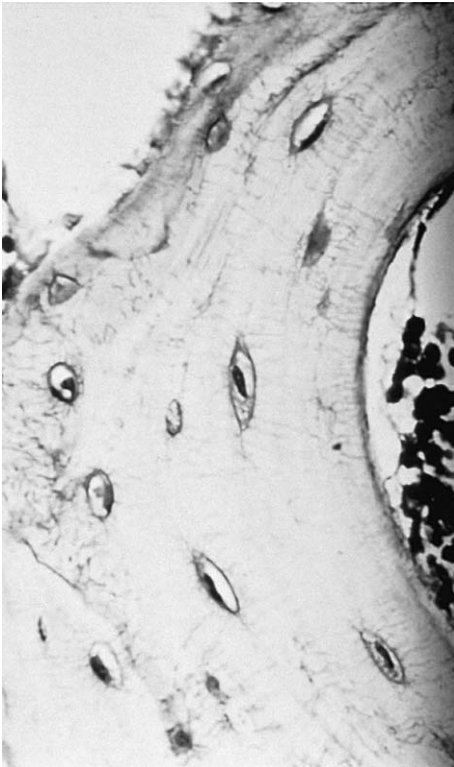


Fig. 2.2.11
Photomicrograph of histological section of a bone trabeculum to show lamellar bone structure.

compressive and tensile stresses. Nature reflects the calculated stress-related bracing seen in some engineering structures such as the Fairbairn crane in which the loading and principal stresses resemble those of the femur (Fig. 2.2.12).

The increased diameter of the bones in the regions of the epiphyses and the presence of subchondral trabecular bone act both to reduce stresses on the articular cartilage and provide a compliant structure to absorb impact loads. Thus any changes in the compliance of the trabecular bone will influence the loading of the overlying cartilage and can indirectly induce changes in the articular cartilage.

Bone as a structure/organ

Adult bone form is determined by a combination of genetic and environmental influences. Fell demonstrated that certain anatomic features of the developing skeleton are formed as a consequence of inherent genetic control whereas others require a functional loading to form.²² An elegant study by Chalmers investigated the development of a mouse femur implanted into the spleen when at the cartilage anlage (precursor) stage of development.²³ This cartilaginous bone precursor developed into a bone with the basic form of the femur but lacked the architectural refinements seen in the normal adult mouse femur. The refinements, such as a waisted femoral neck, cortical thickness, and trabecular architecture, are induced as a consequence of functional loading and are determined by the prevailing loading conditions (Fig. 2.2.13). There is a dynamic interaction between the loads imposed on the skeleton and the morphology of the bones at any point in time throughout life. It is

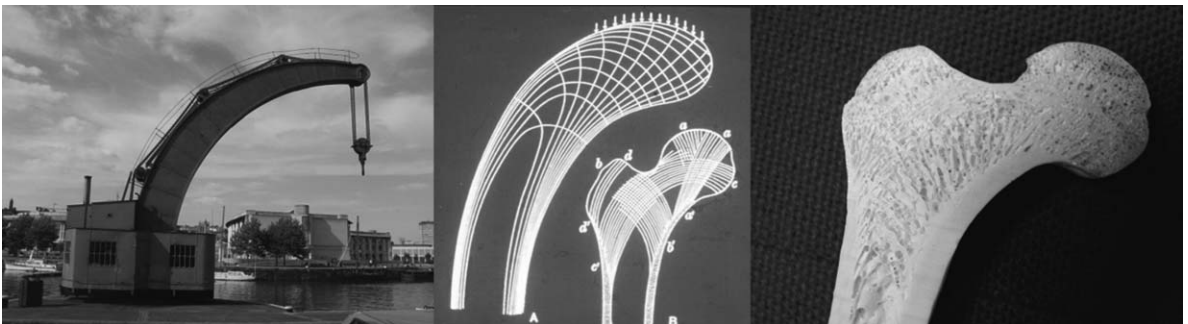


Fig. 2.2.12
Comparison of the structure and stress distribution of the Fairbairn crane and proximal femur.

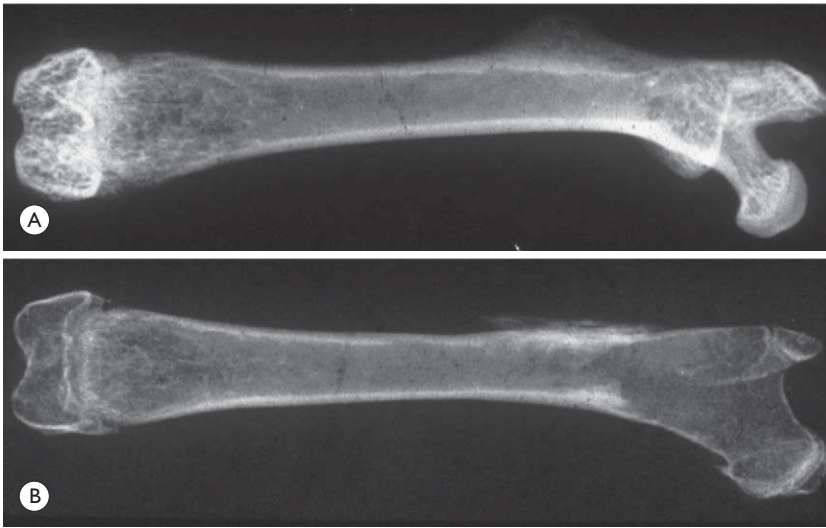


Fig. 2.2.13

Radiographs of mouse femora, showing the adaptations to functional environment (A) from the basic genetic template (B). (Adapted from Chalmers et al, 1962.)

therefore important to appreciate these interactions in order to condition the skeleton for the demands of athletic performance and to understand the modes and mechanisms of failure.

Skeletal development

Growth

Ossification

The skeleton is developed both pre- and post-natally. The majority of the bones of the skeleton, particularly the appendicular skeleton, are pre-formed as miniature replicas in hyaline cartilage. This has the advantage of enabling rapid three-dimensional growth. The material and structural properties of hyaline cartilage are adequate to support the low level of loading prevailing in utero. In preparation for the post-natal changes in loading with gravitational and muscular forces, a more appropriate material must replace the hyaline cartilage. This is accomplished by vascular invasion, calcification and removal of cartilage, and replacement with lamellar bone. This process is endochondral ossification. In a few sites of the skeleton — for instance, the bones of the vault of the skull — bone is formed by direct ossification of a fibrous type I

collagen scaffold. This process is intramembranous ossification.

The bones associated with locomotion are formed by both endochondral and intramembranous ossification. This allows simultaneous longitudinal and circumferential growth of the bones.

Endochondral ossification

The initial vascular invasion of the cartilage replica occurs in the midshaft. In this region the chondrocytes within the lacunae undergo hypertrophy and the extracellular matrix becomes calcified. It is now thought that the endothelial cell invasion occurs in the non-calcified territorial and pericellular matrices following cell death,²⁴ and that cell death in the growth plate occurs through an apoptotic pathway.²⁵ The calcified cartilaginous matrix is then removed by chondroclasts derived from vascular cells and replaced by woven and then lamellar bone. These spicules of bone become strategically organized and form the cancellous bone structure of the metaphysis, termed the primary spongiosa. In human growth this has been suggested by Byers et al as a critical component of the growth process, and the development of the mineralization of the primary spongiosa may be of importance in relation to bone-altering disease in later life.²⁶ These authors also suggest that the secondary spongiosa retains a constant bone mineral volume throughout development. Thus in the developing horse the

mechanical loading from exercise during growth and maturation could affect the level of mineralization of the primary spongiosa.

Simultaneously with the longitudinal growth, the fibrous layer covering the replica bone, the perichondrium, initiates ossification of the fibrous tissue and forms a cuff of bone which develops to form cortices of the tubular diaphysis. This process forms the 'primary' ossification center and is followed by further vascular invasion at one or both ends of the developing bone to form secondary ossification centers. The secondary ossification centers are separated from the primary center by plates of hyaline cartilage known as the growth plate or physis. This process allows for both circumferential and longitudinal growth and enlargement of the bone. The longitudinal growth occurs by utilizing the interstitial growth potential of hyaline cartilage in the proliferative zone of the growth plate. The physis has a distinct structural pattern relating to specific functional activities. The epiphyseal side of the plate is characterized by a resting zone of randomly arranged cells within an extracellular matrix. These cells give rise to the zone of proliferation in which the chondrocyte population is actively dividing. These cells then align into palisades extending toward the metaphysis, with the cells closest to the metaphysis showing hypertrophy of the lacunae. In this zone of hypertrophy the matrix becomes calcified and is removed by chondroclasts. Osteoblasts then deposit woven bone on the calcified cartilage scaffold. This tissue is rapidly remodeled to lamellar bone and forms the trabecular rods and plates of the spongiosa that support the metaphyseal cortex. The width of the physis is increased by appositional growth from the overlying perichondrium. The physis can be described as a discoid physis contributing to the increase in longitudinal growth and width of the metaphysis. The epiphysis also acts as a spheroid physis to increase the size of the epiphysis by radial growth. The early articular cartilage is thick and arranged in a similar pattern to the physal plate with the proliferating chondrocytes close to the articular surface and a radial arrangement of palisades toward the center of the epiphysis (Fig. 2.2.14). The secondary ossification centers and spheroid physes form the cancellous bone that supports the articular cartilage. Abnormalities in the process of ossification can lead to specific orthopedic conditions such as angular deviations of the limbs and osteochondral dysplasias.

The limited thickness of the physis in normal animals allows growth without excessive deformation of the cartilage which would result in angular deformations of the growing bone. In some conditions

where ossification is inhibited and the cartilaginous component of the physis increases in thickness, limb deformities occur during growth. A classic example is dietary rickets. Rapid growth can also result in angular deformities of the long bones.

Intramembranous ossification

As the bones increase in length there is also an increase in overall width of the metaphysis and diaphysis of the bone, accomplished by appositional bone growth on the periosteal surface. In the developing bone a cuff of periosteal bone is formed. The periosteum comprises an outer fibrous layer and an inner osteogenic 'cambium' layer. In this osteogenic layer cells differentiate into osteoblasts which deposit osteoid directly onto collagen type I fibers. This process ultimately forms lamellar bone. In ungulates the type of lamellar bone formed is laminar or plexiform bone. This primary vascular bone is capable of a rapid increase in cross-sectional area. In this type of bone a rapid appositional growth occurs with subsequent infilling of lamellae within the laminae of the vascular network.²⁷ Thus bone growth occurs to the age of skeletal maturity by means of these two processes.

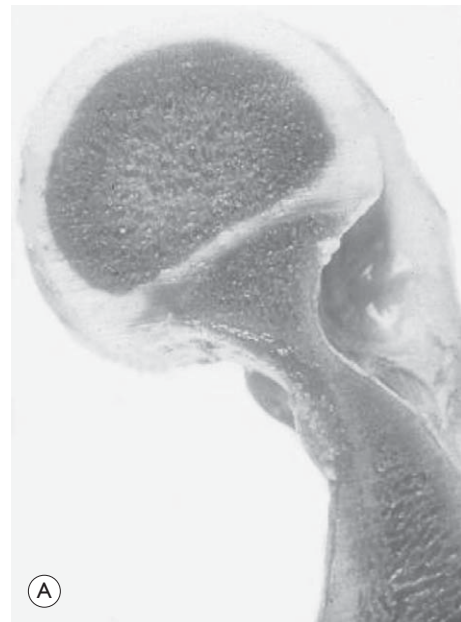
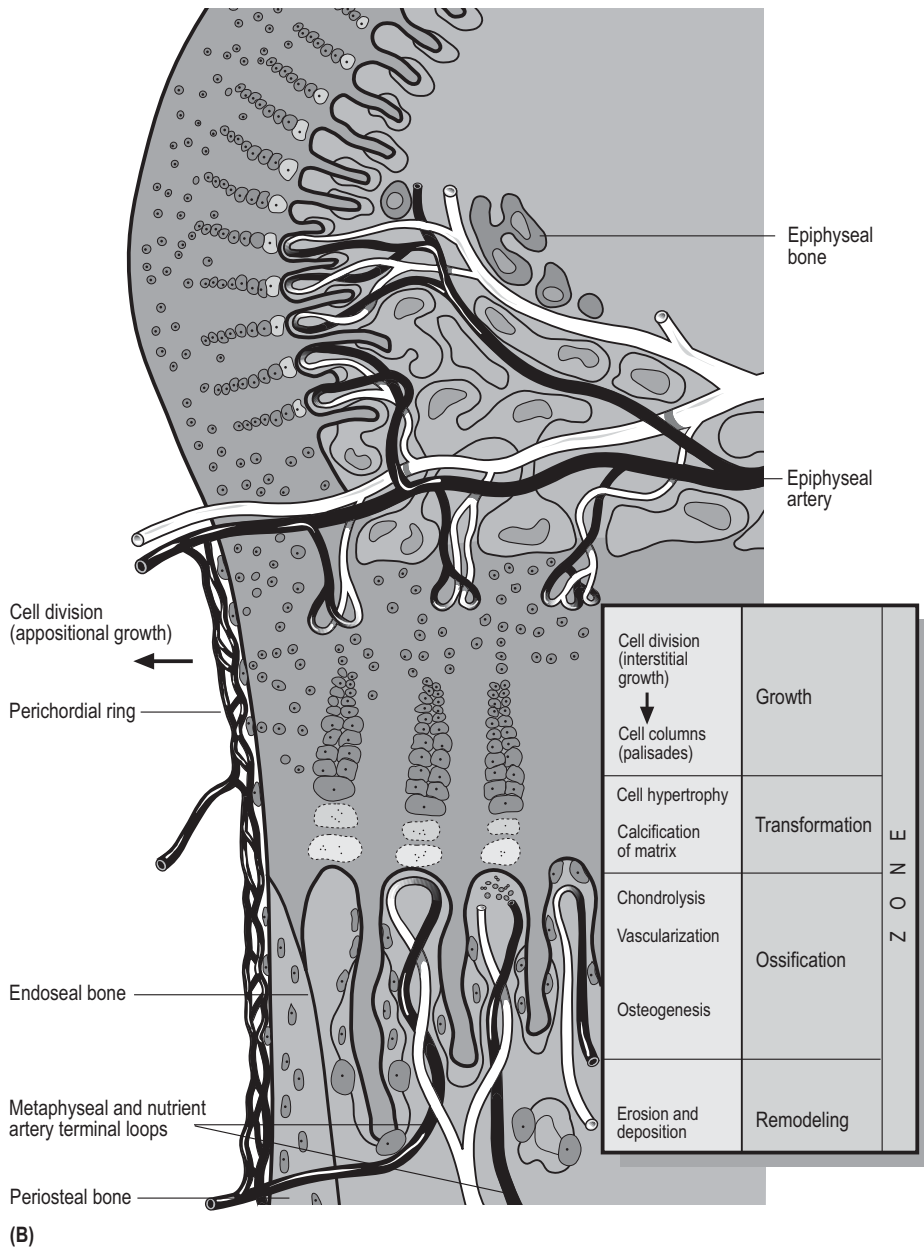


Fig. 2.2.14

(A) The growing spheroid and discoid physes in an immature growing long bone sectioned longitudinally.

**Fig. 2.2.14—continued**

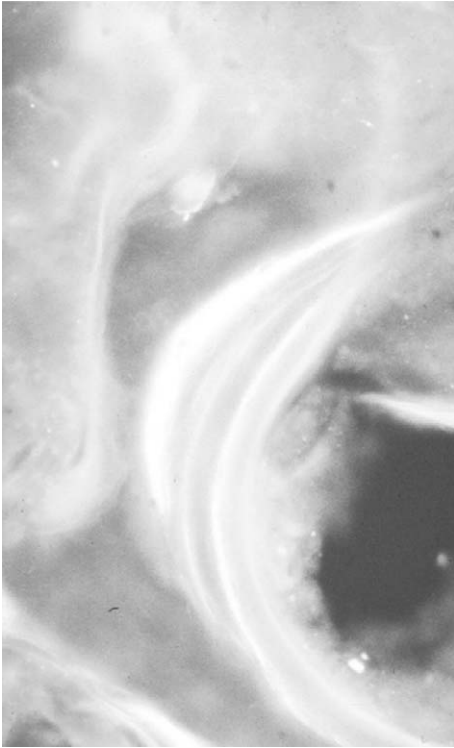
(B) Diagram to show the arrangement of palisades in the two physes.

Modeling and remodeling

During the processes of both bone growth and adaptation to changes in loading, the shape or architecture of the bone is changed by cellular activity to remove and form bone. Unlike cartilage, bone cannot increase in size by interstitial growth; only by these processes of removal and formation of bone surfaces can the

relative proportions of curvatures and the positions of tuberosities be maintained. Changes in shape result from removal and formation of bone at the same time but at different locations; this process is termed modeling and allows changes in three-dimensional tissue space (Fig. 2.2.15).

Modeling is determined both by growth and by mechanical loading. As a long bone increases in

**Fig. 2.2.15**

Photomicrograph of a fluorochrome-labeled section of trabecular bone, showing apposition on one surface indicating a modeling change in architecture.

length the flared metaphysis is narrowed into the diaphysis by bone resorption on the periosteal surface and bone formation on the endosteal surface. In the diaphyseal region increase in bone width during growth occurs by periosteal appositional growth and endosteal bone resorption (Fig. 2.2.16).

Within the bone matrix microdamage can occur as a consequence of repeated loading cycles. Repetitive loading in many inert materials results in accumulation of microdamage and, ultimately, the gross fracture of the structure. Although bone is a structural material, it is living and there are cell-to-cell and cell-to-matrix interactions. Thus changes in matrix can initiate cellular responses to maintain and adjust the matrix. Microdamage is a stimulus to induce osteoclastic resorption of the damaged matrix followed by deposition of lamellar bone by osteoblasts. This dynamic repair process occurs throughout life and during normal functional loading of the skeleton. It is termed remodeling and involves the resorption and removal of bone at the same site but at different points

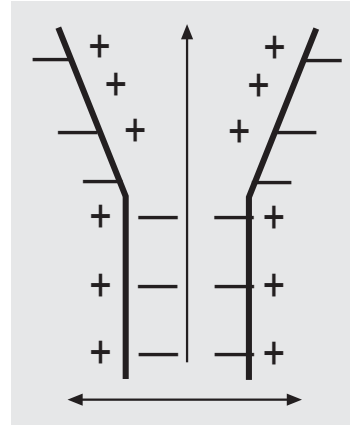
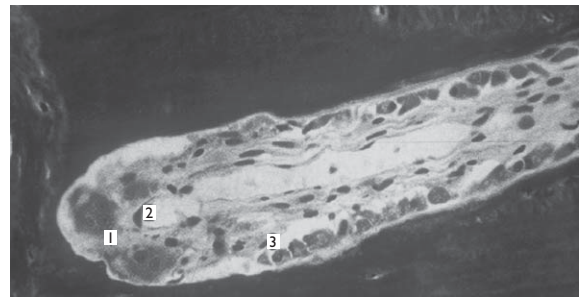
**Fig. 2.2.16**

Diagram of modeling changes at metaphysis and diaphysis during growth.

**Fig. 2.2.17**

A 'cutting cone' of osteoclasts (1) followed by a blood capillary (2) and osteoblasts (3) infilling with circumferential lamellae to form a secondary osteon.

in time (see Fig. 2.2.7). Bone that is formed in remodeling is called secondary bone. These secondary osteons continue to increase in level of mineralization for several weeks after formation of the osteon has been completed. The secondary bone is not as strong as primary bone but is stronger than the damaged matrix. The remodeling process results in the formation of 'secondary osteons'. The initial resorption is effected by a cutting cone of osteoclasts traveling longitudinally within the cortical bone, followed by lining osteoblasts that secrete the new circumferential lamellar bone with entrapment of osteoblasts within the matrix to become the osteocytes within the circumferential lamellae (Fig. 2.2.17). A glycoprotein layer is formed between the newly formed secondary osteon and the adjacent bone tissue which forms the cement line.

Architecture of specific bones

Individual bones have specific morphologic features related to their functional contribution to the skeleton as a whole. The types of bone can be divided broadly into long bones, such as the femur, humerus, radius, tibia, and the metacarpal/tarsal bones; short bones, such as the carpal, tarsal, and distal phalangeal bones; and flat bones, such as the scapula, and some bones of the skull and pelvis. In addition, some bones are not directly involved in the structural support of the body but contribute to the mechanical arrangements of tendons in facilitating the change in direction of tensile forces. These bones, often associated with tendons, are the sesamoid bones, of which the patella, and proximal and distal (navicular) sesamoids are examples.

The long bones can be divided into regions: the epiphyses, metaphyses and diaphysis. These regions show different morphologic arrangements of the bone tissue. The epiphyseal and metaphyseal regions comprise a thin cortical shell of compact bone supported by internal cancellous bone, sometimes termed spongy or trabecular bone. This internal arrangement of bone tissue comprises plates and rods of bone with spaces or cancelli filled with marrow or fat. The bone tissue in these areas often shows a definite pattern of trabecular arcades. This arrangement of internal spongy bone is also seen in the short bones. The geometric pattern is particularly noticeable on radiographs and especially those of the proximal femur and calcaneus (Fig. 2.2.18).

The internal structure of spongy bone has been of interest to scientists, engineers, and surgeons since the time of Galileo. In the 1890s comparisons were made between the pattern of cancellous bone and analysis of stress trajectories in engineering structures. The proximal region of the femur with the femoral neck and head is loaded in a manner very similar to that in engineering structures like the Fairbairn crane, and the trabecular architecture reflects the lines of principal tensile and compressive stresses calculated for these structures (see Fig. 2.2.12).

Recent developments in technology have allowed the distribution of principal strains to be measured in the living skeleton and the data from such studies have provided supporting evidence that these bone trabeculae are strategically placed to optimize the strength of the structure with minimal mass of tissue. In animals this is important to reduce the energetic cost of locomotion. The thickness and structural



Fig. 2.2.18
Radiograph of the proximal human femur to show trabecular arcades.

density of trabecular bone also reflect the magnitude of the loads taken in that region of the bone. This has been shown in training studies; for example, the trabeculae in the dorsal region of the third and radial carpal bones are thicker than those in the more palmar regions in horses that have been trained for peak athletic performance. These observations can be used in diagnosis of bony changes related to mechanical loading of the skeleton. The site-specific changes, such as those in the carpal bones, are often found to be related to predilection sites of skeletal injury and pathology so knowledge of these interactions can enhance diagnostic and prognostic clinical skills.

The arrangement of bone tissue in the diaphyseal region of long bones is markedly different. This region is similar to a tube; hence the term tubular bone. The wall of the tube comprises compact cortical bone, of differing thickness, and a medullary cavity containing blood vessels, marrow, and fat. This arrangement of bone reflects a different mechanical loading pattern; whereas in the short bones the loads are predominantly compressive loads, in the long bones spanned by tendons and ligaments there are large bending and torsional moments in addition to compressive loading.

Again, the biologic drive is to reduce the energetic costs of locomotion, whilst ensuring adequate structural strength to avoid failure during physiologic loading levels. In engineering design these

requirements to provide maximum strength in bending and torsion using a minimal mass of material are met by a wide-diameter, thin-walled tube. This essentially places the material at the greatest distance from the neutral axis, where there are neither tensile nor compressive stresses. In long bones the medullary cavity represents the average neutral axis relative to the loading pattern of the bone. Thus strength is maximized and mass minimized to reduce the energy costs associated with moving the limb. In animals evolved for high-speed locomotion there is a high biologic drive to reduce energy costs. In engineering structures the material type, mass, and distribution all contribute to the ability of the structure to resist functional loads. However, to allow for occasional overload a safety margin is normally incorporated into the design, such that the structure will not fail as a consequence of occasional overload. Studies have shown that the skeleton also incorporates a safety margin, although this is not uniform in relation to skeletal location. The neck of the femur in humans is known to be able to withstand about five times bodyweight. The disadvantage of a safety margin is the additional mass of material. This becomes significant in the more distal regions of the limb since additional mass increases the momentum of the swinging limb and this in turn would result in a significantly increased energy requirement in decelerating and accelerating the limb, particularly in high-speed equine athletes. Currey, analyzing data from Vaughan & Mason, showed that a higher incidence of fractures during racing occurred in the more distal bones of the limb and hypothesized that this could be explained by a lower safety margin in the more distal limb bones.³

Although the diaphysis of long bones is essentially tubular, the distribution of bone in terms of cortical thickness may be non-uniform around the circumference of the bone. This reflects different levels of resistance to bending in different planes and the associated distribution of bone mass.

Factors controlling shape and size

Genetic influences

Both between species and within species, the variation in the specific geometry of individual bones is a consequence of two main factors: first, a genetic component that will control a basic bone mass, and second, a response to prevailing mechanical conditions which will modify the basic genetic pattern in relation to

both the magnitude and the distribution of loads applied to the particular bone.

Disuse will result in a tendency toward the genetic basic bone mass. Abnormal loading will induce a concomitant abnormality in the size and shape of the bone. Chalmers in 1962 demonstrated these controlling factors by transplantation of the cartilage precursor of the mouse femur to the spleen, where it developed with adequate blood supply but devoid of mechanical input.²³ Although the basic shape of a femur could be recognized, this bone lacked the refinements in both mass and architecture exhibited by the normal functionally located bone in littermates (see Fig. 2.2.13). Further evidence that certain anatomic features of specific bones were genetically predetermined and others were formed as a consequence of local stimuli was provided by Fell using isolated limb buds from developing embryos in organ culture.²² Genetic modification of a basic anatomic skeletal pattern is often seen in relation to attaining specific conformation for either visual or functional requirements. Even within specific breeds of horse certain genetic strains are evident and linked to differences in bone mass and architecture. Other factors influenced by selective breeding, which could be described as genetic modification, such as muscle mass, may have an indirect influence on bone mass as a consequence of increased levels of mechanical loading of the bones. As both a tissue and a structure, bone has the property of functional adaptation.

Environmental influences

Other factors such as nutrition and hormonal environment may also influence the ability of a bone to attain the full genetic potential. However, one of the most potent environmental influences on bone mass and architecture is the prevailing mechanical environment.

Functional loading of bone refines the inherent genetic form. The levels of mechanical input can vary throughout life with an associated response in terms of skeletal adaptation.

Both during growth and maturation and in the skeletally mature adult, bone responds to mechanical loading. The relationship of the pathophysiology of functional adaptation to training and conditioning regimens in general and in the horse in particular is important in maximizing performance and minimizing skeletal injury. In determining the aspects of functional loading that influence the bone cell populations and consequently are important in controlling the

architecture of the skeleton, it was essential to be able to quantify the functional environment of the skeleton.

Although techniques were available to measure strains on cadaveric bone, using photoelastic coatings, stress coat, and electronic strain gauges, it had not been possible to quantify the effects of loads on the living skeleton until the cyanoacrylate tissue adhesives became available. In the early 1970s Lanyon & Smith²⁸ and van Cochran²⁹ made the first measurements of strain in the living skeleton. The ability to bond foil rosette strain gauges to living bone allowed both the magnitude and distribution of the principal strains on the surfaces of a number of bones to be measured during activities such as locomotion. From these measurements it was possible to determine the loading patterns of different specific bones. For example, the radius in quadrupeds is loaded in bending with principal tensile strain aligned to the long axis of the bone on the cranial cortex and principal compressive strain aligned to the long axis of the bone on the caudal cortex,¹⁶ whereas the tibia showed a significant component of torsional loading.³⁰ The physiologic loading pattern of these long bones can be used in determining the appropriate fixation methods to treat fractures in these bones.

Strain distributions on the surface of bones with defined internal trabecular arcades again provide confirmatory data to show the strategic alignment of the trabeculae of spongy bone with the directions of principal tensile and compressive strains.³¹ This technique increased the understanding of the response of bone to changes in mechanical demands, a mechanism called functional adaptation.

Functional adaptation of bone

General principles

The bony skeleton provides structural support for the body throughout life and also enables locomotion by virtue of the linked skeletal elements forming a system of rigid levers upon which forces can be exerted by muscles through tendons and ligaments. Articulations between the individual bones allow energetically efficient movement. The basic genetically determined bone mass is optimized for energetic efficiency and to accommodate the loads imposed. Thus throughout life as the activities change, the process of functional adaptation enables the appropriate adjustments to be

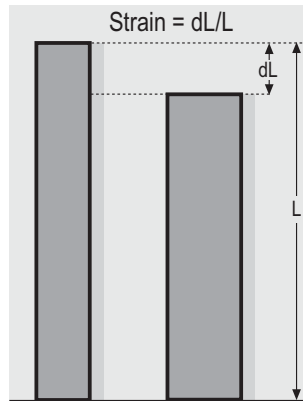


Fig. 2.2.19
Diagram to show deformation or 'strain'.

made to the mass and architecture of the skeleton. The biologic signals that induce bone cell activity and control the adaptive process are related to the deformation of the tissue as a consequence of the loads applied by muscles and gravity. Deformation is termed 'strain' and is the change in length in relation to the original length (Fig. 2.2.19). Although the response of bone to mechanical loading has been known for some time, the ability to quantify imposed changes in mechanical environment of the skeleton and relate these to the consequent biologic response of the bone has only been possible using the technique of *in vivo* strain measurement.

Dynamic strain similarity

By applying strain gauges to the living skeleton of a variety of species, from fish through reptiles, birds, and mammals, including the horse and human, the level of bone deformation during peak physiologic activity has been shown to be remarkably similar.³² Thus it was hypothesized that bone had evolved in this wide range of species to optimize to a threshold level of deformation, irrespective of histological structure. A simple strain-controlled feedback loop was proposed. If an increase in loading resulted in an elevated strain above the threshold value, this triggered the bone cell populations to synthesize bone matrix to increase the mass of bone and thus for the new loading conditions to reduce the increased levels of deformation back to the threshold values. In the event of a decrease in loading and consequent level of deformation, the bone cells would respond by activating a net bone resorption and decrease bone mass.

Optimization of mass and architecture (Wolff's concepts)

In the event of a persistent change in the normal pattern of loading, the bone strain patterns would be altered and this persistent change in strain distribution would initiate a redistribution of bone through a modeling response to alter the architecture of the bone as a structure. Such changes may involve structural optimization by changes in both the distribution and mass of bone.

Early studies in which the strain on the skeleton was increased and an adaptive response was evoked supported the theory of a strain control feedback mechanism. The characteristics of the mechanical osteogenic stimulus were elucidated by a series of studies in which bones were subjected to known strain characteristics and the consequent osteogenic response was measured. These studies were of two basic types: those in which a defined mechanical regimen was imposed on a bone following surgical intervention and those in which an exercise regimen was applied to the intact skeleton. Increased loading of parts of the skeleton induced by removal of adjacent bone or by attachment of loading devices to isolated bones allowed the imposition of a specific strain environment. The early studies inducing increased loading by removal of an adjacent bone and consequent overloading of the remaining ones by normal locomotion demonstrated an adaptive response to increase the overall cross-sectional area and thus restore the strain levels to the functional optimal values, thus supporting one of Wolff's hypotheses.^{33,34}

Osteogenic mechanical stimuli

The nature of the mechanical stimulus that elicited an osteogenic response was investigated by a more controlled study of specific mechanical variables and their influence on adjustment of bone mass and architecture.

Such studies involved the use of implants attached to the bone and used to impose controlled mechanical stimulation. These experiments elucidated some of the osteogenic aspects of the strain environment. During normal strain distributions, such as those occurring during walking, the level of osteogenic response, in terms of increased cross-sectional area of bone, to imposed strains was found to show a high correlation with the rate of bone deformation.³⁵ This suggests that

training using a normal type of exercise requires a high loading rate to elicit an osteogenic response. A more refined method of studying the exact nature of mechanical signals that stimulate bone formations was developed by using isolated segments of bone. Ideally an organ culture might be used for such studies but culture of bone is difficult in terms of culturing a significant mass of true ossified tissue and the absence of the hormonal regulation and its interaction with mechanical cues precludes such models.

Using isolated bone models *in vivo*, it was possible to define in a more specific manner the strain characteristics that induced bone formation.³⁶ The effect of numbers of cycles of loading applied on a daily basis was investigated, together with the effect of magnitude of the imposed strain. It was shown that there was a 'dose-response' effect with strains below the normal physiologic threshold inducing bone resorption and a consequent decrease in cross-sectional area and those above the physiologic threshold showing an increase related to strain magnitude. From these experimental studies a number of mathematical models were derived in which it appeared that there was a window of strain magnitude that retained bone mass with neither resorption nor formation. This was termed the 'lazy zone'.³⁷ Strain magnitudes outside this zone induced either bone resorption or bone formation. Interestingly, Rubin & Lanyon investigated the relationship between the number of loading cycles imposed on a daily basis and the resultant effect on bone mass in terms of bone mineral content and cross-sectional area.³⁶ This work showed that a maximal effect on bone mass could be attained with only 36 cycles of loading at 0.5 Hz each day. Any additional cyclical loading did not attain a greater effect on bone mineral density or cross-sectional area. Bone mass was shown to be maintained with as few as four loading cycles per day, whereas complete cessation of loading resulted in a decrease in bone, predominantly by endosteal resorption leading to a reduced cortical thickness, together with some increase in cortical porosity. This study suggests that short periods of osteogenic cyclical deformation can induce a maximal adaptive response. In terms of conditioning bone in equine athletes this might be interpreted as a short period of daily trotting. The stimulus appears to require cyclical deformation as the application of constant load did not induce bone adaptation.³⁸

Hillam & Skerry developed a non-invasive method of loading the rat forearm and used this model to demonstrate that mechanical loading could modify the normal modeling patterns seen during growth.³⁹ A

short period of cyclical loading changed a bone resorption surface to a bone-forming surface. The applied loading induced an aberrant strain distribution. This model was also used by Mason et al in determining the gene activation patterns associated with mechanical loading and putative signaling molecules in the transduction and integration of mechanically induced bone remodeling.⁴⁰ Forwood & Turner used a similar approach by applying a bending moment to the rat tibia and confirmed the response to high strain rate cyclical deformation in a mammalian model. This suggests the principles can be extrapolated to other species, including the horse.⁴¹

Thus the principles of these findings have implications for the training of young horses using a diverse exercise regimen. Perhaps on the basis of this evidence, horses that are used for competitive events that involve movements outside the natural range on a frequent basis should be introduced to such exercises at an early age when the architecture of the skeleton could be influenced to adapt to these loading demands and thus resist damage and failure in adulthood. Events such as jumping, dressage, trotting, racing, and pacing may be candidates for such practice. However, any training regimens must be introduced over an adequate time period with a graded increase to accommodate adaptation and avoid damage.

Rate of adaptation

Adaptation to loading in bone can be initiated by short periods of cyclical loading. Therefore, to prolong this type of exercise or to introduce long periods of such exercise too rapidly, particularly on a hard surface, may lead to induction of microcracks and ultimately the gross fracture of the bone. Damage even to the delicate internal architecture of the trabecular bone with subsequent stiffening, particularly of subchondral regions, may reduce the absorption of high-impact loads and inflict damage upon the overlying articular cartilage (Fig. 2.2.20).⁴² Mechanical factors may thus initiate a cascade of events leading to degenerative joint disease. The joint must be considered as an organ and changes to one component tissue or structure will impact on the whole joint.⁴³ Interestingly, Chen et al observed differences in heel strike transients between races with different incidence of degenerative joint disease so it is possible that conformation may influence the transmission of impact transients in equine limbs and predispose to degenerative joint disease.⁴⁴

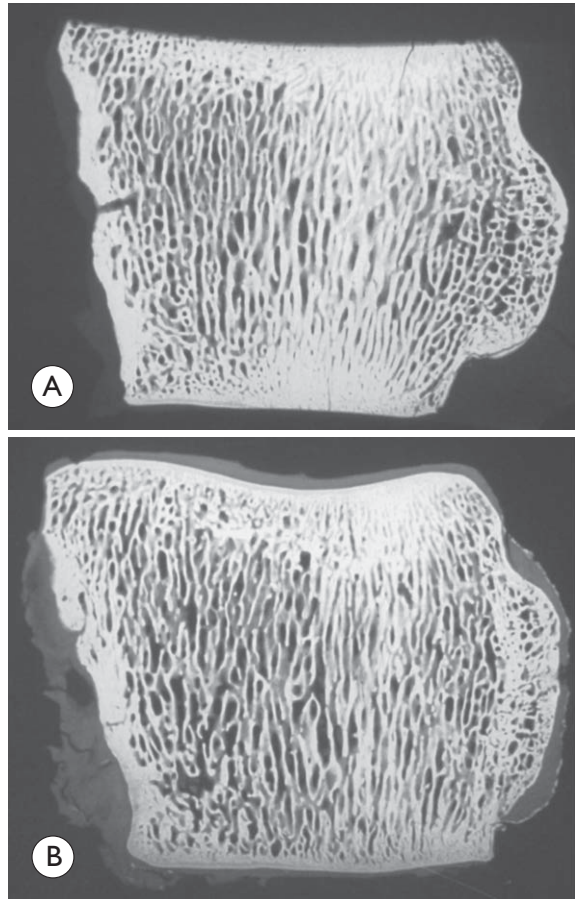


Fig. 2.2.20

Photomicroradiograph to show localized adaptive hypertrophy of trabecular and subchondral bone to imposed exercise (A) compared to control (B).

This localized adaptive hypertrophy of trabecular bone can be seen following imposed exercise regimens: for instance, the dorsal regions of the third and radial carpal bones.⁴⁵ Furthermore, with an osteogenic exercise regimen given for only a short period each day, these localized bone changes occur extremely rapidly.⁴⁶ This localized change in loading can induce a change in both bone and cartilage, the osteochondral unit. Increased exercise in the adult horse results in a thickening of the trabecula of the subchondral bone, a thickening of the subchondral plate, and a thickening of both the calcified and hyaline layers of the overlying articular cartilage.⁴⁷ Experimental studies have also shown the sensitivity of cancellous bone to changes in mechanical loading and these data can be indirectly related to training methods in the horse.⁴⁸

These morphological changes result in a change to the mechanical properties of the microstructure which can be measured by indentation techniques⁴⁹ and micromechanical testing of single trabeculae.⁵⁰ The adaptation within a bone in relation to a very specific pathway of load transmission through the bones of the skeleton is seen particularly well in the carpal bones where significant differences are found between the dorsal and palmar regions of these bones. Long-term loading with associated stiffening of the bone in the dorsal region may relate to the common occurrence of cartilage fibrillation and breakdown in this region.

Microdamage

Horses that are given this type of training regimen, especially if the training is imposed over a short period of time, show the clinical signs of 'bucked shins'. If the training is based upon a structured increase in duration of short periods of exercise that induces high bone strain, the bone will adapt and the physiologic adaptation will condition the bone for athletic exercise rather than a pathophysiologic response leading to 'bucked shins'. This response is also seen in racing greyhounds and can lead to overt fractures.

Microcracks

Accumulation of microcracking within the bone matrix results in a remodeling response in which the secondary bone has inferior properties to primary bone. Crack formation is seen as a fractal pattern of matrix damage (Fig. 2.2.21). Microcracks have been demonstrated in several species of athletic individuals such as the horse^{51,52} and the racing greyhound.⁵³ Cracks can be observed by bulk staining the bone prior to histological sectioning in order to confirm that the cracks are not processing artifacts. The cracks can be seen as stained defects within the matrix (see Fig. 2.2.21). When the level of magnification is increased these defects in the bone matrix can be seen to continue at an ultrastructural level (Fig. 2.2.22). This damage to bone matrix through which the delicate processes of the osteocytes pass can potentially alter the interaction between cell and matrix and cell-to-cell communication. Processes such as generation of streaming potentials by movement of ionic fluid

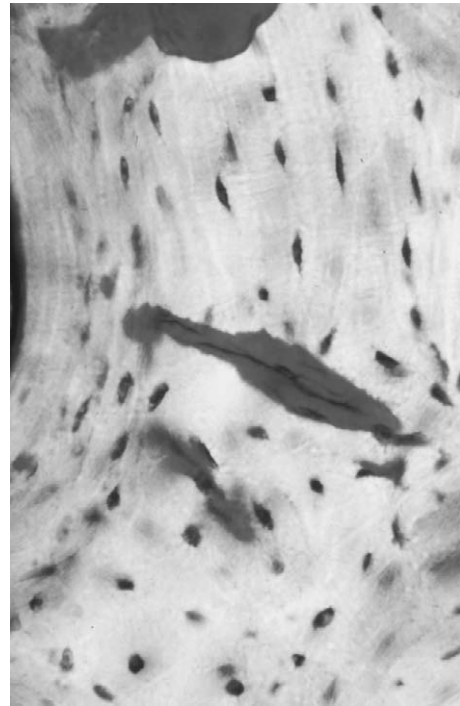


Fig. 2.2.21
Photomicrograph of a section of cortical bone stained to show a microcrack within the matrix.



Fig. 2.2.22
Scanning electron micrograph of a microcrack in bone matrix, showing the fractal pattern of damage.

through the macro-, micro-, and nanoporosities of bone will be altered. These changes may be the cause of the known response to microdamage such as apoptosis and osteonal remodeling. Experimental studies using the non-invasively loaded rat antebrachium

have demonstrated that a rapid application of osteogenic cyclical loading will lead to a change in structural stiffness, indicating a plastic post-yield deformation of the bone. The bone will fracture if such loading is continued. However, if bones treated in this way are examined histologically a short time after loading has stopped, an increased porosity is seen as osteoclastic cutting cones target the intracortical damage. A predominance of secondary osteons is found on examination after a longer period of time.

Once primary bone has been remodeled, the bone type has been permanently changed. These changes, seen in the experimental model used by Bentolila et al, are closely related to those seen in studies on the induction of sore/bucked shins using a high volume of high strain rate exercise.⁵⁴ The effect on subsequent adaptation and failure of highly remodeled bones compared to those in which a gradual adaptation of primary bone has been induced is not really known. Epidemiological studies on racetrack fractures and relation to training methods are few. One such study by Wood et al (personal communication) has shown that certain types of training regimen are associated with a higher-odds risk of fatal failure related to commencement of training at an earlier age. Short bursts of high-speed training in the months before racing reduces the fracture risk (Parkin, personal communication). This appears to agree with marker data showing increased bone formation with short periods of fast work.⁵⁵ Also Boston & Nunamaker⁵⁶ report the advantages of short-distance breezing rather than long-distance gallops in reducing 'bucked shins'.

Osteocyte apoptosis

In addition to the overt fracture of the matrix at the ultrastructural to gross level, there is an effect of this type of loading-induced damage on the cellular component of the bone tissue. Osteocytic apoptosis rates are modified and some effects are also seen on gene expression.⁵⁷ Interestingly, estrogen treatment also modulates osteocyte apoptosis.⁵⁸ Recent observations by Ehrlich et al that mechanical cues to bone cells are mediated through the estrogen receptor may explain this commonly observed response.⁵⁹

Remodeling

The consequence of exercise-mediated microdamage through a rapid imposition of cyclical mechanical

loading is seen either as overt fracture or, more often, as an increase in formation of secondary osteons to limit crack propagation and replace damaged matrix. However, secondary bone has been shown to be inferior in terms of material properties and may compromise mechanical properties of the bone as a structure.

Thus the avoidance of undue secondary osteon formation may prevent the reduction of the mechanical properties of the overall bone structure. Controlled osteogenic exercise during training would induce a more gradual adaptive response and increase the bone mass with minimal damage of the matrix, thus preserving the mechanical properties of the skeletal structure and reducing the risk of catastrophic failure. Training regimens that appear to optimize bone adaptation without matrix damage comprise short periods of high-intensity exercise.

In physiologic loading, exercise at a high rate of deformation was found to be a potent osteogenic stimulus.^{46,56} However, strain distributions that differed from those experienced during normal physiologic activities may elicit osteogenic responses at subphysiologic strain magnitudes. It has been suggested that these represent an 'error' signal which, if only imposed occasionally, does not lead to modeling changes; however, a repeated imposition of these unusual strain distributions can induce structural changes in bone architecture. An example is seen in the serving arms of professional tennis players where bone mass can be approximately 30% higher than in the non-serving arm.⁶⁰ Thus an abnormal gait, particularly if inducing high strain rates, will induce an osteogenic response.

In the horse the level of bone deformation increases as a function of locomotor speed. Thus, as the speed of the walk increases so too does the level of bone deformation. This is also seen in the trot, with an extended trot leading to abnormally high rates of bone strain. In the horse, as speed increases there is normally a change in gait which is controlled by energy efficiency drives; the levels of oxygen increase with speed but there is an optimal speed at each gait for oxygen utilization. At speeds beyond the optimal, the utilization of oxygen becomes less efficient and this leads to a gait transition and further optimal levels of oxygen utilization for the new gait.⁶¹ In addition, at the gait transition the level of bone deformation is reduced.⁶² This may represent a means of controlling bone mass and also reducing damage due to fatigue loading of bone.

Safety factors

As with most structures, the skeleton can withstand occasional overloads. In designing a structure such as a bridge there is a built-in safety factor to accommodate unforeseen overload. The level of overdesign is related to a balance between risk of overload and cost of materials. The architecture of the skeleton has been shown to follow very similar principles to those seen in engineering of structures. An excess of material would minimize risk of structural failure but carry a high cost in terms of energy requirements. These factors may explain the findings that bones toward the proximal region of the limbs have a higher safety factor than those at the distal extremity. For instance, the human femur is able to withstand loads of five times normal bodyweight.

Site-specific fracture incidence

In the horse, an animal evolved for high-speed locomotion, the energy costs of additional mass at the extremity of a long limb, acting like a swinging pendulum, would be high. Currey analyzed the incidence of fractures in race horses reported by Vaughan and showed a higher incidence in the more distal bone of the limb where safety factors would be reduced to decrease energy costs.

Exercise studies in horses have shown a reduced level of functional adaptation in the more distal bones together with an increase in fracture toughness. This may represent a different mechanism to limit crack propagation and consequent fracture.⁶³

Gross fractures

As with any structure, a single overload can result in a monotonic failure. Since bone exhibits functional adaptation the mass and architecture are adapted to meet the prevailing magnitude and distribution of load. However, a single excessive load that is greater than the given safety factor will result in failure. The type of failure in terms of damage of the structure at a gross and microscopic level will depend upon the energy and velocity of crack propagation.

When the bones are subjected to load distributions different from those to which the bone has adapted, failure can occur at much lower levels. This type of failure may be seen when limb movements are

uncoordinated, such as during recovery from anesthesia or at the end of periods of strenuous activity when muscles are fatigued and limbs loaded asymmetrically (Dow, personal communication).

The risk of fracture may also be increased if a new type of exercise is introduced suddenly without an appropriate period of training for both muscle and bone adaptation.

Training influences on bone adaptation

In general there has been a significant lag between scientific advance and change in the training methods used in the equine world in general and in the horse-racing industry in particular. No doubt some of the biologic mechanisms controlling adaptation of bone to its functional environment could and should be applied to the conditioning of equine athletes. It is also probable that the empirical traditional methods of training do incorporate some scientific principles as a consequence of many years of experience. However, given the highly competitive nature of the equine industry, it is difficult to identify training methods that relate to high and low incidences of injury. Recently a number of epidemiological studies have been performed to identify both the relationships between specific types of training and incidence of bone fractures. The response of the bony skeleton to different quantified training regimens has also been monitored using the minimally invasive techniques of blood markers for assessing bone formation and bone resorption.

Monitoring of skeletal responses to training

The ability to make an objective study on the monitoring of adaptive changes in the skeleton to training requires two components: first, the ability to define and quantify the training input, and second, to measure the effect on the bones of the skeleton directly or indirectly. Even in the laboratory these are difficult to achieve; in field studies direct measurements such as radiography, scintigraphy, and dual-energy X-ray absorptiometry (DEXA)/computed tomography (CT) scanning are not practical. Therefore, indirect assessments of bone modeling and remodeling are being developed. Blood markers have been used both in controlled studies and in commercial training facilities. In studies using controlled exercise regimens it is possible

to relate these to changes in marker levels and to evaluate factors such as seasonal, age, and gender-related effects.

Markers of bone adaptation

In a controlled exercise study over 1 year in 2-year-old Thoroughbreds, the effects of the exercise on two biochemical markers of bone formation were determined. These markers were the carboxy-terminal propeptide of type I collagen and the bone-specific alkaline phosphatase. In addition, one potential marker of bone resorption, the pyridinoline cross-linked telopeptide domain of type I collagen, was also monitored. In both the low- and high-intensity exercised groups there was a significant reduction in marker levels over the year. This is expected as a normal age-related change. However, it was encouraging that the pattern of reduction differed between groups in a way that indicated an increase in bone turnover in the high-intensity trained group.¹⁸ These results are encouraging, as they indicate the potential to develop a non-invasive method of monitoring the effects of training on bone.

Direct monitoring of skeletal adaptation

Radiographic techniques

Radiography can be used to visualize some direct responses of the skeleton to increased or decreased mechanical demands. Changes in the architecture and radiographic density of bones can be used to monitor the competence of the skeleton. Standardized positioning and the use of a calibration step wedge allow standardization of exposure by optical digitization, which may then allow a determination of relative bone density in relation to responses to applied training or conditioning regimens. Radiologic methods have been used to assess functional adaptation to exercise in the equine skeleton and a radiographic index has been used to assess the changes in shape of the third metacarpal bone in response to training. Studies have been performed to define the limitations and level of accuracy of this technique that indicated acceptable accuracy, providing alignment of the limb, cassette, and X-ray machine is accurate. These findings suggest that the radiographic index can be used to measure MC3 bone shape using relatively simple and widely available technology.⁶⁴

Peripheral quantitative computed tomography (pqCT)

Improvements to radiographic methods can be obtained by the use of pqCT. A two-dimensional image showing bone mineral density distributions may be generated and quantitative information on cortical cross-sectional area and second moment of area can be provided (see Fig. 2.2.9). Thus the effects of training could be monitored in relation to specific regimens. The disadvantage of this technique at present is that, as in magnetic resonance imaging (MRI), the machine is fragile and the horse has to be anesthetized. Also the aperture of the machine is limited and thus only small peripheral limb segments can be assessed. For experimental studies and in young horses, this technique can provide considerable useful data on the response of specific distal limb bones to imposed exercise.

Dual energy X-ray absorptiometry (DEXA)

This system can be used to determine bone mineral content of the skeleton and regions of the skeleton. These scanners are normally used in assessment of bone density in relation to metabolic bone disease, particularly post-menopausal osteoporosis. The disadvantage in relation to monitoring of equine skeletal responses to training is the need to anesthetize the horse, together with the limited area that can be scanned. In humans it is possible to relate individual scan data to population values and provide a fracture risk score. This may be an interesting concept in relation to monitoring of the equine skeleton but is not yet possible.

Nuclear scintigraphy

By attaching an isotope to a bone-seeking bisphosphonate, areas of active bone modeling or remodeling can be labeled. By imaging with a gamma camera these active sites can be located in the living skeleton at specific points in time. Although used predominantly for diagnostic purposes, this technique could identify site-specific skeletal responses to exercise or mechanical loading.

There is a need for an effective non-invasive method to monitor both bone quality and skeletal integrity during development and in both athletic training and competition. Some handheld methods have been tried. Potentially the use of ultrasound in terms of measuring speed of sound through bone and broad-band

attenuation can give information on both modulus and structural integrity. The systems are available commercially for human use and also recently for use in the horse. A combination of this type of monitoring and associated adjustment of training schedules could prevent the overt occurrence of conditions such as 'bucked shins'.

Training strategies to condition the skeleton of the equine athlete

Age-related changes in the skeleton

In a study on fatigue strength, Nunamaker et al measured surface bone strains on the third metacarpal bone of young and old North American Thoroughbred horses to determine the mechanisms involved in fatigue failure of bone leading to fracture.⁶⁵ They found greater levels of strain (deformation) at the gallop in the young horses with almost a 40% reduction in strain in the older horses. The significance of this is that the fatigue failure point of a bone depends upon both the magnitude of the deformation and the number of loading cycles to the failure point. Thus if the strain magnitude is reduced there can be a greater number of cycles of deformation prior to failure. In addition, since bone is a dynamic tissue that responds to mechanical deformation, the bone architecture as a structure can be modeled to reduce the bone strain at high loads.

These authors suggest that changes in shape of the third metacarpal bone during growth and maturity may represent a mechanism to reduce strain and mitigate fatigue failure. The high incidence of fatigue failure, as seen in 'bucked shins' in young horses subjected to exercise that induces high strains over short periods of time, would support this hypothesis. Interestingly, Nunamaker et al also investigated breed differences in the incidence of 'bucked shins', hypothesizing that there might be a breed difference in the material properties of the bone matrix leading to a difference in the fatigue failure patterns.⁶⁶ They determined fatigue properties in the bone material of the third metacarpal bone in Thoroughbred and Standardbred horses. No significant differences were found in the mechanical properties of the bone. This may suggest that other factors such as the training methods and type of loading affect the incidence between these

two breeds. Perhaps this is indicative that imposed mechanical events, even within a breed of horse, can influence the incidence of such conditions. An understanding of the pathobiology and material properties of bone could be used to determine the appropriate age and exercise regimen to minimize such injuries.

Exercise during development

Most of the training and conditioning of equine athletes occurs after skeletal maturity. In some cases, such as training of race horses, the period of training is relatively short and the ongoing training is also limited to short periods. The training methods are largely empirical based on a traditional approach rather than advances in the science underlying training.

Recently a number of studies have been investigating the concept of introducing conditioning for athletic performance during the period of post-natal growth and development. This approach imposed the distribution of mechanical loads associated with the athletic activity at a time when modeling processes are active in forming the functionally related aspects of skeletal structure. Thus optimization of primary bone development can be attained in relation to the loading patterns that will be experienced during athletic activity. There is some evidence that bone strain can be reduced with gait transition, decreasing by 42% at the trot/canter transition.⁶² Thus in activities where gait changes are suppressed as a component of the particular athletic activity — for example, in dressage and in racing trotters and pacers, the high strains at high speeds may predispose to microdamage when introduced in the adult. However, if these patterns of gait are introduced gradually during the growth and modeling phase of skeletal development, then appropriate skeletal architecture can be attained with lower risk of damage. This, combined with the incremental increases in training load during development, will also increase skeletal mass and may reduce the strain magnitudes that would occur if training were introduced after skeletal development, and thus minimize injuries.

Changes in foot balance in foals have also been shown to induce strain changes of up to 40% reduction in medial metacarpal compressive strains and 100% increase in lateral compressive strains. However, these were found to return to normal distributions after a period of time.⁶⁷ As discussed previously, bone adapts to increased strain and changed strain

distribution, so a return to normal strain at a local site may reflect an adaptation resulting from changes in mass and distribution of bone. In this study changes in bone mass or architecture as a consequence of the changed loading were not reported. This adaptation to abnormal loading during growth may result in an abnormal architecture of the bone with possible implications for other skeletal components such as joints in later life. Therefore, attention to exercise type, intensity, and foot balance during growth and development should optimize the skeleton for athletic activity in the adult. The concept may also apply to other skeletal tissues in which the process of growth can be modified by early conditioning.

Human athletes are frequently identified as children and both train and compete during growth and maturation. In addition, human athletes tend to undertake high volumes of training. The equine athlete, particularly the race horse, is given relatively low volumes of training and spends considerable time in the stable.

Conclusion

Horses as equine athletes must be conditioned and trained to optimize the whole animal for the particular type of athletic activity. An extremely high proportion of all injuries in the equine athlete in general and in the race horse in particular are associated with the musculoskeletal system. Within musculoskeletal injuries, fractures and bone-related pathology account for a great proportion. Bone, as both a tissue and a structure, is acutely responsive to mechanical loading and thus can be conditioned to withstand the applied loads. In order to maximize performance and minimize injury it is important to appreciate the pathobiology of functional adaptation of bone. This, together with appropriate monitoring, can enable owners, trainers, and veterinarians to apply the science underlying functional adaptation to the training of specific equine athletes, thus improving equine welfare.

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Tendon and ligament physiology: responses to exercise and training

Roger K.W. Smith & Allen E. Goodship

Introduction

Role and definition of tendons and ligaments

The distinction between tendons and ligaments has been largely an anatomic one — tendons join muscle to bone whereas ligaments join bone to bone. Although this division is generally true, topographic, biomechanic, ultrastructural, and matrix compositional investigations have revealed a merging of these two structures in a continuum from ‘pure’ tendon to ‘pure’ ligament.

In connecting muscle to bone, tendons have been considered rather inert structures that are involved in the movement of joints. However, while this ‘positional’ function is still an important one, certain tendons in cursorial animals have developed another role in acting as springs to store energy for efficient locomotion. This is particularly true in the horse, where the tendinous structures on the palmar aspect of the metacarpal region — the superficial digital flexor tendon (SDFT) and the musculus interosseus medius tendon (suspensory ligament, SL), in particular, and the deep digital flexor tendon (DDFT) — act to support the hyperextended metacarpophalangeal joint during weight bearing (Fig. 2.3.1), releasing energy when the limb is protracted (Fig. 2.3.2). Hence, at fast gaits, the horse effectively bounces up and down on springs, similar to a child’s pogo stick. As further modifications to this role, the digital flexor tendons have accessory ‘ligaments’, which attach the tendon directly to bone to provide a direct bone-to-bone tendinous connection when under full weight bearing.

It would be expected, and indeed found, that these tendons would have mechanical characteristics for this function as a spring, while tendons with a more ‘traditional’ positional role (e.g. human digital flexor tendons and the common digital extensor tendon (CDET) of the horse) would require stiffer characteristics. Up to a twofold difference in structural stiffness has been found between equine digital flexor and extensor tendons.¹

Periarticular ligaments, while having similar composition to tendons, are anatomically more complex, with multiple bundles of fascicles that frequently spiral and are taut and relaxed at different joint positions, depending on the fascicle bundle. In addition to their role in providing support for the joint, they also provide proprioceptive information. The fibrous joint capsule is usually closely associated with these periarticular ligaments and functions in a similar way. Consequently, fibrous joint capsule is also an ‘honorary’ ligament.

Incidence of injury

Strain-induced injury of the tendons and ligaments is the most common orthopedic injury in athletic animals, be they equine or human. Recent epidemiologic surveys of race-horse injuries sustained at UK racetracks between 1996 and 1998 showed that of all limb injuries (82% of all incidents), almost one-half (46%) were due to flexor tendon and/or SL injuries.² As observed clinically, this study confirmed that these injuries were much more common in older horses racing over jumps (steeplechasers or hurdlers) than in younger race horses racing on the flat. Furthermore,



Fig. 2.3.1
Galloping horse, showing the hyperextended metacarpophalangeal joint under weight bearing.

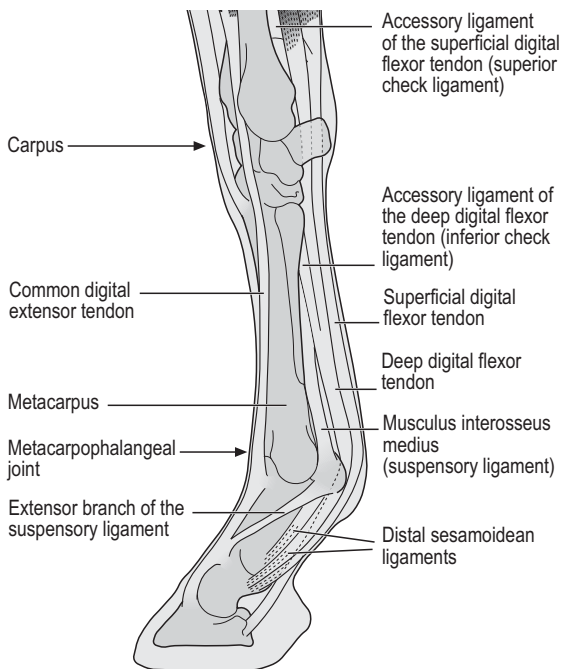


Fig. 2.3.2
Topographical anatomy of the distal limb of the horse.
(Adapted from Adam's Lameness in horses, 3rd edn, reproduced with permission.)

the data from these flat races correlated well with previous data published from studies in the USA (0.760 per 1000 starts in 1992).³ However, this epidemiologic data, being obtained from only those injuries occurring during racing, represents just the tip of the iceberg. More recent studies investigating the incidence of superficial digital flexor tendinitis in horses

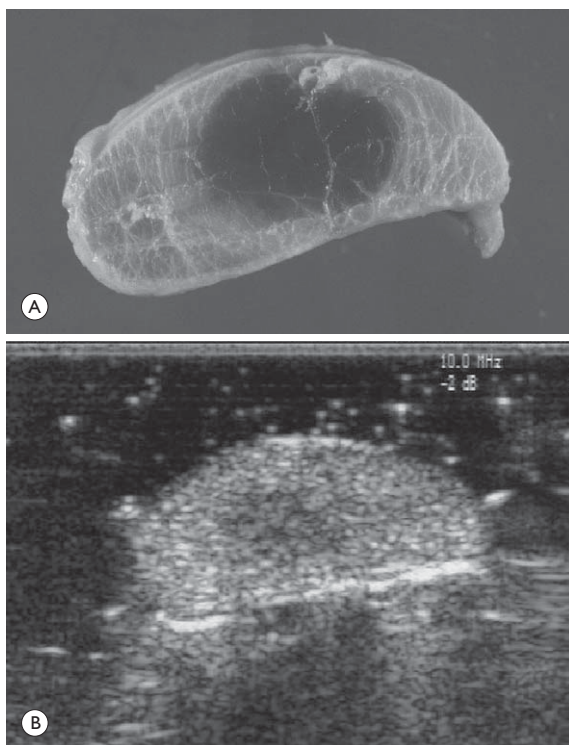
during training showed that almost one-half (43%) of horses had evidence of tendon pathology, and that this incidence increased with age.⁴ These data correlate well with similar ultrasonographic studies evaluating Achilles tendon damage in professional human athletes.⁵ Other data recorded for training and racing injuries in flat race horses in Japan showed not only a rising incidence from 6.39% for 2-year-olds to 17.43% in 5-year-olds, but also, more worryingly, a rising overall incidence from 1990 to 2000 (Oikawa & Kasashima, personal communication). The reason for this is unclear but, interestingly, the incidence of Achilles tendinopathy in man has doubled in incidence in Europe in the last 10 years, believed to be associated with greater levels of exercise and increased longevity.^{6–8}

The incidence in non-race horses and for other strain-induced injuries of other tendons and ligaments has not been reported, although all horses involved in athleticism appear to be highly susceptible to tendon and ligament injuries. An interesting exception is ponies, which rarely suffer from superficial digital flexor tendinopathy, although they do have a relatively high incidence of desmitis of the accessory ligament of the DDFT.

Pathogenesis of tendon injury

Tendons and ligaments can be injured in one of two ways — overstrain or percutaneous penetration/laceration. The latter will not be considered further in this chapter. Overstrain injuries are believed to occur by one of two mechanisms. They can result from a sudden overloading of the structure, which overwhelms its resistive strength. This type of injury is probably the mechanism for most ligament and some DDFT injuries in the horse. However, for the most common strain-induced injuries in the horse, involving the palmar soft tissue structures of the metacarpal region, the clinical injury is believed to be preceded by a phase of degeneration. The evidence for this preceding degeneration is based on four observations:

1. The identification of 'asymptomatic' lesions, both grossly and microscopically, in post-mortem studies of normal horses.^{9,10} Care, however, should be made in distinguishing gross central discolorations in otherwise normal tendons, which are more likely to represent early clinical injury than the preceding degeneration as compositional analyses reveal some opposite changes to that seen induced by

**Fig. 2.3.3**

(A) Central discolored region of 'asymptomatic' superficial digital flexor tendon (SDFT) — these 'lesions' are probably more representative of subtle clinical injury than the degenerative stage of tendinitis because they can be visualized ultrasonographically in a water bath as core lesions characteristic of clinical injury (B).

exercise (see below) and many of these lesions can be identified ultrasonographically (Fig. 2.3.3).

2. Many, if not all, clinical strain-induced tendinopathies are bilateral with one limb more severely affected than the other. Careful ultrasonographic examination will reveal changes in the contralateral limb in many cases. In those seemingly unilateral cases, blood-flow studies have demonstrated increases in the 'normal' contralateral tendon, which would suggest that it is not totally unaffected.¹¹
3. Epidemiologic studies have demonstrated close associations between age and exercise, and tendon injury.^{2,4}
4. Following on from these epidemiologic observations, experimental investigations have demonstrated evidence of degeneration associated with a

synergistic action of both age and exercise (see below).^{12,13}

Thus, degeneration is usually the first phase of tendinopathy. This can be likened to 'molecular inflammation',¹⁴ which does not provoke a repair process, as after clinical injury, but rather progressively weakens the tendon. Any change in the structural properties of the tendon does not have to be great as the tendon is already operating close to its tolerance limit. Clinical injury occurs when the highest stresses encountered by the tendon overwhelm its structural integrity, resulting in irreversible damage. Once this occurs, the damage created induces a repair process characterized by inflammation followed by fibroplasia (scar tissue formation).

This process allows the incorporation of risk factors that have been identified for tendinitis. These risk factors act to increase the peak loads on the SDFT, thereby increasing the risk of structural disruption. One of the most important factors is the speed of the horse. The faster the horse is going, the greater the risk of tendinitis.² Thus, hard going is associated with tendinitis as it increases the speed of the horse and also increases the peak impact loading.² Slower surfaces (including soft going) tend to be protective. Other factors, such as the weight the horse is carrying, fatigue, and the shoeing, can all influence peak tendon loads in this way. Low heels were thought to be protective of tendinitis as this conformation tends to increase the load in the DDFT, a secondary supporter of the metacarpophalangeal joint, thereby reducing the support necessary from the SDFT and SL. However, as the highest loading in the DDFT is towards the end of the weight-bearing phase, this may not have an influence on the initial high-level loading of the SDFT at the onset of weight bearing.

Once the peak load on the tendon overcomes its structural strength, there is physical disruption to the tendon matrix. This varies in degree from fibrillar slippage, with breakage of cross-linking elements, to fibrillar rupture and, in some cases, complete separation of tendon tissue. This damage initiates a reparative sequence of events not dissimilar to that in other soft tissues, such as skin, which is characterized by phases of inflammation, followed by fibroplasia (see Tendon injury and repair, p. 124), which results in the replacement of normal tendon tissue with scar. With the formation of abundant scar tissue, the healed tendon becomes strong but it is functionally inferior to normal tendon. As a structure, healed tendon is stiffer than normal tendon, which compromises the

tendon function and predisposes to reinjury, often at sites adjacent to the original injury.¹⁵

Structure of tendon

Biology of the muscle — tendon — bone unit

The musculature in the limb of the horse is proximally positioned to minimize the weight of the distal limb. This enables the horse to achieve efficient high-speed locomotion through increased stride length and reduced energetics of limb protraction. This means that the tendons associated with these muscles have to be long to traverse the joints on which they act and, consequently, the digital flexor tendons, for example, are some 45 cm in length.

The horse's distal limb has reduced the number of phalanges to a single digit (with vestigial digits, the splint bones, either side of the third metacarpus), which has simplified the tendon and ligament anatomy (see Fig. 2.3.2). The distal fore- and hindlimb therefore has an arrangement of three palmarly positioned structures — SDFT and DDFT with their associated accessory ligaments, and the SL — which serve to support the hyperextended metacarpo-/metatarsophalangeal joint, and two or three dorsally positioned digital extensor tendons, which function only to extend the distal limb during protraction. Hence the loads experienced by these tendon groups are very different — the extensor tendons experience only low loads while the palmar tissues are subjected to high loads of weight bearing (7–10 kN on the SDFT).¹⁶

The horse has further adapted the basic muscle–tendon–bone unit in order to withstand the high weight-bearing loads and for its palmar soft tissue structures to act as an elastic unit for energy storage and efficient locomotion. Thus, the superficial digital flexor muscle has a much larger amount of connective tissue within it and has an accessory ligament (accessory ligament of the SDFT; proximal, or superior, check ligament) that 'bypasses' the muscle belly to insert on the distal radius. These two adaptations allow the musculotendinous unit to withstand greater loads than would be possible by the muscle itself. The SL has taken this adaptation even further. It was originally derived from a muscle and still contains a variable amount of muscle within its mid- and proximal regions. It could therefore be classified as a structure in which the tendon component has replaced the

muscular tissue. However, its cellular morphology more closely resembles a ligament and, in the absence of a significant muscle belly, is usually classified as a ligament.

The muscle belly of the SDFT is not redundant, however, although its proposed role is different from the classical function of flexing the limb. Recent data have suggested that this muscle serves to fix the origin of the tendon. In vitro maximal contraction of the muscle has demonstrated a maximum of only 2 mm of muscle shortening.¹⁷ It is hypothesized that the muscle acts to dampen the high-frequency damaging peak loads when the foot is placed on the ground at high speeds. Thus, loading of the SDFT can be considered an essentially passive process where energy is stored by the elastic properties of the tendon and returned when the limb flexes.

As a further adaptation to the long tendon length and the hyperextended metacarpo-/metatarsophalangeal joint, the digital flexor tendons are contained within synovial sheaths in regions where they pass over high motion joints (the carpal sheath proximally and the digital sheath distally). While these structures protect the tendons from shear damage, they limit the ability of the tendons to heal, both because they are bathed in a synovial environment and because the thick surrounding layer (paratenon) of extrasynovial tendon is absent within the confines of a tendon sheath. The paratenon is believed to be important in supplying fibroblasts capable of repairing tendon after injury.

Tendon receives its nutrients from three potential sources — intratendinous blood supply emanating from the musculotendinous junction and its osseous insertion, from blood vessels entering the tendon via mesotenon attachments within tendon sheaths or the paratenon, and from the synovial fluid within the tendon sheath. The relevant importance of these components depends on the tendon and tendon site. For the metacarpal region of the SDFT, studies have suggested that the intratendinous supply is the most important,¹⁸ as necrosis was only achieved by ligation of the blood vessels within the tendon whereas stripping the paratenon had no effect.

The blood flow in the SDFT has been recorded as between 1 and 2 mL/min/100 g,^{10,11,19} which is of similar magnitude to that within resting skeletal muscle. Blood flow has been shown to increase twofold with exercise,¹¹ although this can be delayed in horses that have not been trained. Injury caused an even greater increase in blood flow (>300%), which has been recorded in both limbs of a horse with clinically

unilateral tendinitis, further confirming the bilateral nature of the disease.

Tendon as a connective tissue

Although seemingly homogeneous on initial gross inspection, tendon is, in fact, composed of a complex arrangement of extracellular proteins in which are embedded cells, blood vessels, lymphatics, and nerves.

Tendon cells

Little is known about the cells that populate equine tendon. Although they are collectively known as tenocytes, they are unlikely to be a uniform population of cells because they differ considerably in nuclear morphology on light microscopy and when grown in culture. Previous descriptions have described three types,¹⁰ although a fourth type is evident within the endotenon tissue (Fig. 2.3.4; Table 2.3.1), and in other species, the synovial cells lining the outside of the tendon are differentiated from those within the tendon.²⁰

The function of these cell types is not known but their location, morphology, and presence in young or adult tendon propose functions which are reflected in the type classification (see Table 2.3.1). The actual synthetic activity of these different types is unknown because there are currently no markers for identifying each type, nor methods for selectively recovering them from tendon tissue. Tenocytes have been often likened to fibroblasts and, while they have many similarities, it is unlikely that all these tenocyte types are identical to the fibroblasts seen in the skin or scar tissue. Recent studies have shown that fibroblasts recovered from different tissues behave differently in terms of the

amount of certain proteins they synthesize (Smith & Heingard, unpublished data).

Work performed on laboratory animal²¹ and equine (Ralphs, personal communication) tendons has shown that tenocytes have a large number of cytoplasmic extensions, which connect to neighboring cells via gap junctions. This provides a syncytium that could

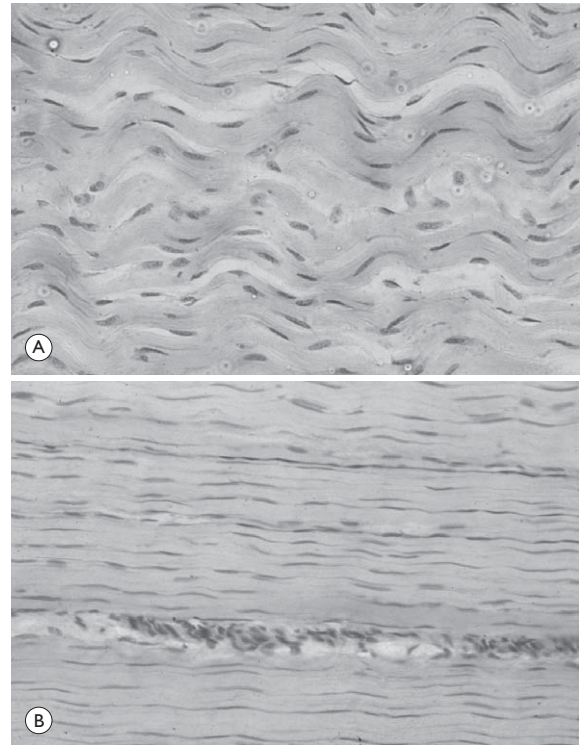


Fig. 2.3.4

Hematoxylin and eosin histologic sections showing the different morphologies of tenocyte nuclei in young (A) and mature (B) equine tendon. Note the large numbers of cell nuclei within the endotenon in the mature tendon.

Table 2.3.1 Types of cell within tendon and ligament based on nuclear morphology and location

Cell classification	Type	Nuclear morphology	Location
I	'Resting'	Spindle-shaped nuclei lying between collagen fibers	Within the tensional region of all adult tendons
II	'Active'	Cigar-shaped nuclei lying between collagen fibers	Within young tendon and both young and adult ligaments
III	'Chondrocytic'	Round nuclei	In compressed regions of tendons and areas of chondroid metaplasia
IV	'Precursor'	Round nuclei with prominent nucleoli	Endotenon

provide an efficient system for mechanotransduction, similar to that occurring between osteocytes in bone.

Tendon matrix

As the cellular component of tendon is small, the functional properties of tendon rely on the extracellular matrix. This is determined, in turn, by both the composition and, equally importantly, the arrangement of these proteins within the matrix. It is constructed from a series of increasingly sized subunits into a hierarchic arrangement (Fig. 2.3.5).

Morphology

Crimp

'Crimp' refers to the characteristic waveform seen in longitudinal tendon histologic sections (Fig. 2.3.6). Originally suspected to be a preparation artifact, it is now known to be a real feature of tendon fascicles that is responsible for the 'toe' region of the stress-strain curve for tendon where tendon elongates with only a low level of applied stress (Fig. 2.3.7). It is thus eliminated within the first 2% of tendon elongation and hence is unlikely to be present when the tendon receives normal weight-bearing load when standing. However, the 'toe' region has important implications for the overall strain capabilities of the tendon. Crimp can be described by its angle and length and these change as the animal ages (see p. 120).

Ultrastructural morphology

Collagen fibrils, the basic 'building block' of tendon, appear as banded filament on transmission electron

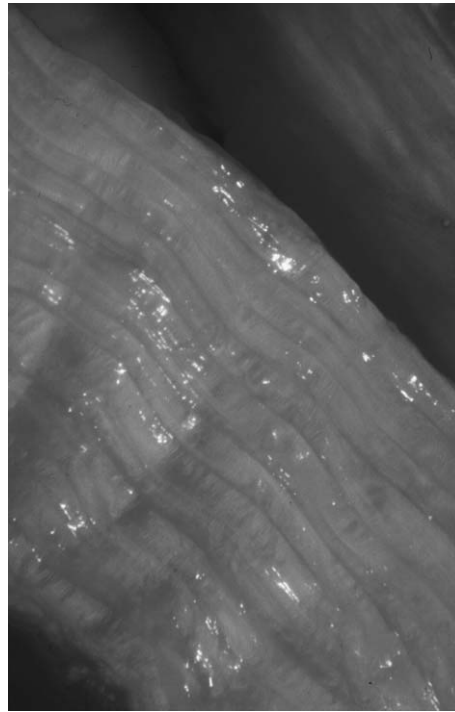


Fig. 2.3.6
'Crimp' pattern seen in the fascicles of the accessory ligament of the deep digital flexor tendon.

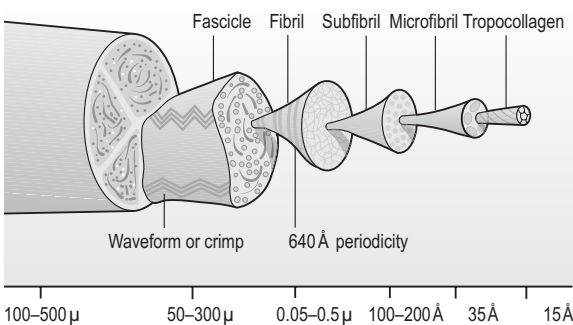


Fig. 2.3.5
Hierarchic arrangement of collagen in tendon. (Courtesy of the Veterinary Clinics of North America: Equine Practice, reproduced with permission.)

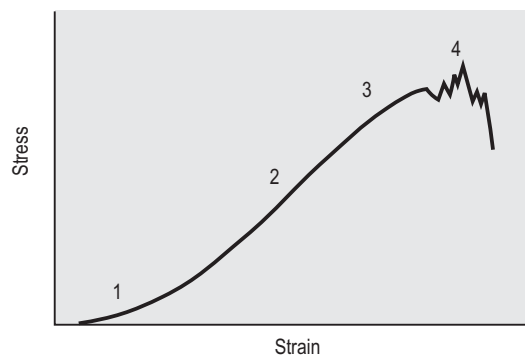


Fig. 2.3.7
Stress-strain curve for tendon. Zone 1 refers to the toe region, thought to be associated with the elimination of crimp; zone 2 to the linear phase from which the elastic modulus is calculated; zone 3 to the yield point after which irreversible damage occurs; and zone 4 to where individual tendon fibers rupture leading to complete failure. (Courtesy of the Veterinary Clinics of North America: Equine Practice, reproduced with permission.)

microscopy of longitudinal sections of tendon. Transverse sections show the collagen fibrils as electron-dense circular structures of varying sizes. The distribution of collagen fibril diameters can be determined from these sections, as well as assessment of the mass average diameter, which takes into account the relative proportion of the area taken up by different-sized fibrils.

Investigations into the development of tendon in laboratory animals have shown that collagen fibrils aggregate, resulting in thicker collagen fibrils. Recent work has proposed a regulatory mechanism for this

process where only unipolar (N–C terminals) collagen fibrils can fuse, resulting in a bipolar fiber (C–C terminals).^{22,23} Thus, as the tissue matures, the proportion of unipolar fibrils becomes depleted, limiting the final size of the collagen fibril.

Whereas this process might also occur in the early development of equine tendon, the picture is much more complicated after birth. The adult SDFT has a bimodal distribution of fibrils with large numbers of small fibrils and lower numbers of large-diameter fibrils²⁴ (Fig. 2.3.8). Furthermore, there are differences between tendons, with the DDFT having fewer

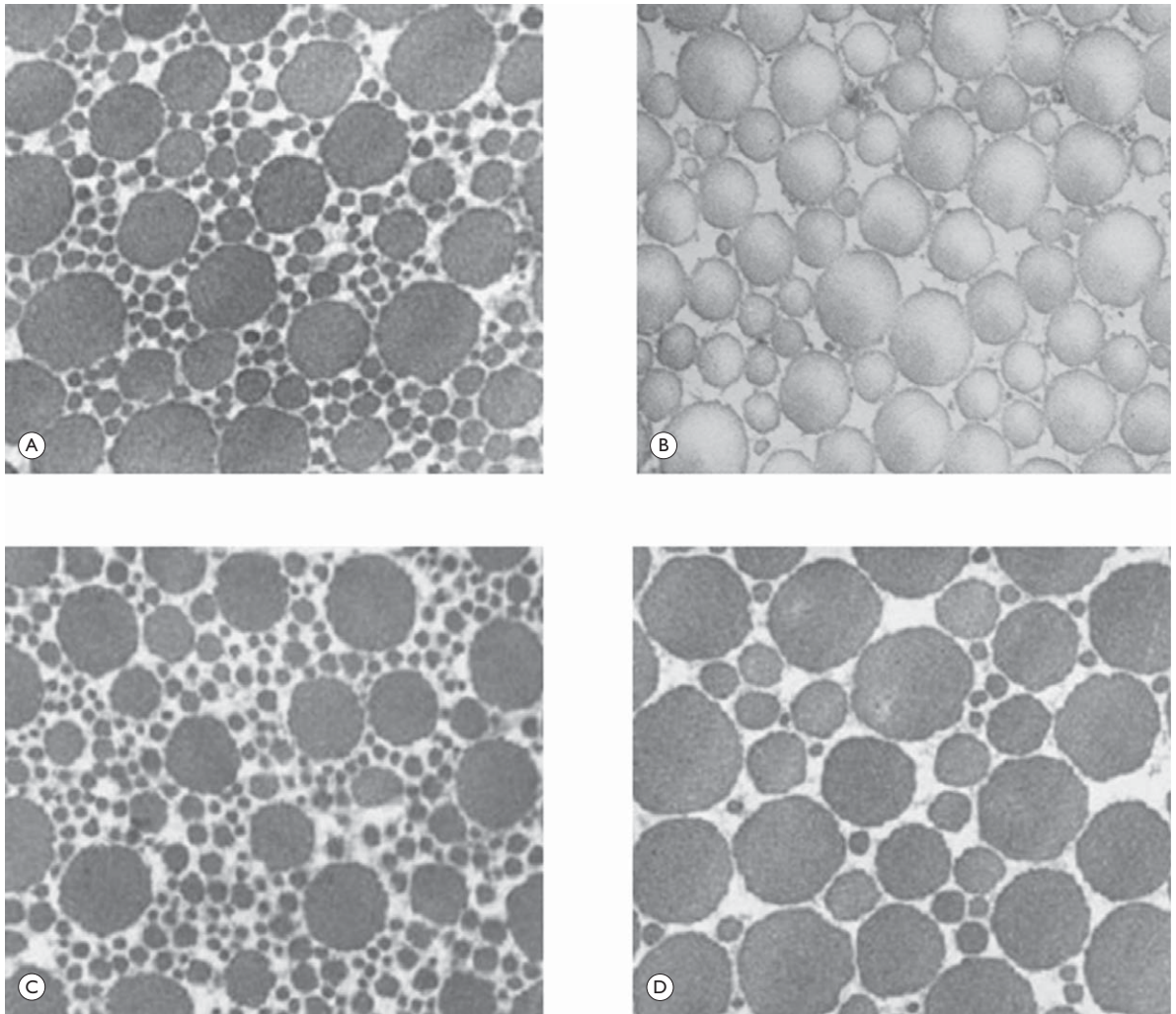


Fig. 2.3.8

Collagen fibril morphology in (A) the superficial digital flexor tendon, (B) the deep digital flexor tendon, (C) the suspensory ligament, and (D) the common digital extensor tendon. Note the similarities of the primary supporters of the metacarpophalangeal joint (superficial digital flexor tendon), and the positional tendon (common digital extensor tendon) with the deep digital flexor tendon.

small-diameter and greater numbers of large-diameter fibrils.²⁵

Molecular composition

Tendon is predominantly composed of water, which makes up approximately two-thirds of the weight of the tissue. The presence of this water is fundamental to maintaining the elasticity of the tissue because dehydration results in an increase in stiffness and tendons containing less water tend to be stiffer (Birch, personal communication). Although unsubstantiated at present, movement of water through compartments within the tendon might result in 'streaming potentials', which could provide a mechanism for mechanotransduction, whereby mechanical forces on the tendon influence the metabolic activity of the tenocytes.

The remaining third of the content of tendon (the dry weight) is predominantly composed of type I collagen. This protein is a major component of all connective tissues. Each type I collagen molecule is constructed within the endoplasmic reticulum of the cell from two $\alpha 1$ (I) chains and one $\alpha 2$ (I) chain, which forms a triple helix with non-helical N and C terminal extensions (propeptides) and is known as a procollagen molecule. These individual collagen molecules are assembled by cleavage of their propeptides at the N and C terminal ends by specific N- and C-proteinases, either after secretion into the immediate pericellular environment or intracellularly (Kadlar, personal communication). This results in a tropocollagen molecule that is 285 nm long and 1.4 nm wide. These molecules are then assembled into collagen fibrils in a highly organized fashion, each collagen molecule overlapping its neighbor by a quarter length (the 'quarter stagger' arrangement that is responsible for giving the banded pattern seen in electron microscopy), so that five collagen molecules make up a subunit of the collagen fibril. Although collagen molecules will spontaneously self-assemble, other proteins are likely to play a role in orchestrating this assembly (see below).

The fibrils are stabilized by the formation of covalent cross-links between lysine/hydroxylysine residues in adjacent fibrils, catalyzed by the enzyme lysyl oxidase. The collagen fibrils are in turn assembled in a longitudinally oriented pattern into increasingly larger subunits, which ultimately form the collagen fibers seen under light microscopy. These collagen fibers are further associated into tendon fascicles,

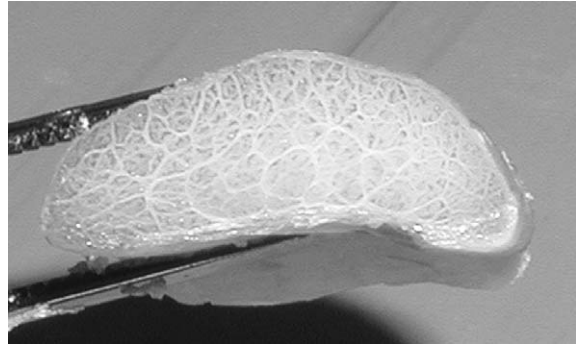


Fig. 2.3.9

The cut surface of the superficial digital flexor tendon (SDFT), showing the fascicular arrangement.

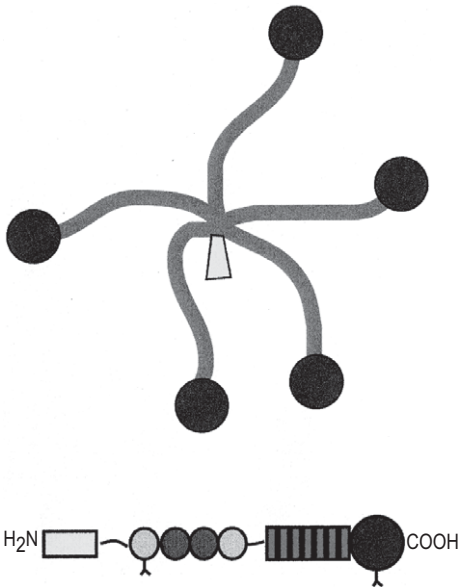
which can be identified on the cut surface of a tendon (Fig. 2.3.9).

Non-collagenous components

While 80% of the dry weight of tendon is composed of collagen, the remaining 20% (5–6% of the wet weight of the tendon) comprises a wide variety of non-collagenous proteins. Although these are only a small component of the tissue, recent work has suggested they are vital for the organization and function of the tissue.²⁶

During growth of the digital flexor tendons, one of the most abundant proteins is cartilage oligomeric matrix protein (COMP). This protein consists of five 'arms', joined at their N termini and with globular C termini (Fig. 2.3.10). There are only low levels of this protein in tendon at birth but it accumulates during growth within the digital flexor tendons with the highest levels achieved in the tensional region of the SDFT at skeletal maturity in the horse (at approximately 2 years of age) of approximately 10 mg/g wet weight.²⁷ This equates to approximately 30 mg/g dry weight or approximately 1% of the wet weight. After the period of growth, however, levels of COMP in the tensional, but not the compressed, region of the SDFT fall (see p. 121).

The function of COMP has not been completely determined but it is known to bind fibrillar collagens (including type I) via a zinc-dependent mechanism.²⁸ This interaction with collagen occurs via the globular C terminal domains at four equally spaced sites along the collagen molecule.²⁹ The size of the arms is such that it cannot interact with more than one site on one

**Fig. 2.3.10**

A cartilage oligomeric matrix protein (COMP) molecule. (Courtesy of Dr K Rosenberg and Comparative Biochemistry and Physiology, reproduced with permission.)

collagen molecule but instead can order five collagen molecules for assembly into a collagen fibril during the earliest stages of collagen fibril formation. As it does not bind to formed collagen fibrils, COMP is displaced from the fibril. This makes its role as a structural protein within the formed tendon matrix a difficult one to rationalize. However, recent studies have shown that COMP can accelerate collagen fibrillogenesis *in vitro* and it is possible that this protein acts more as an 'organizational' molecule rather than in a structural role (Heinegård, personal communication). This would be supported by observations that the highest levels are present during the growth of the tendon when matrix is being synthesized and that levels fall after skeletal maturity when there is little change in the structural properties of the tendon. While possibly not important for the structural integrity of the matrix once it has formed, it is potentially critically important in the formation of soft tissue collagenous matrices that are designed to withstand loads, as it is present only in tendon, ligament, cartilage, intervertebral disk, and meniscus. Indeed, initial data relating mechanical properties of SDFT at skeletal maturity to COMP levels demonstrate a significant positive relationship.²⁶ Thus, the hypothesis is

advanced that the more COMP synthesized during growth, the stronger the resulting tendon. In further support of this function, a functional 'knockout' of the protein caused by a naturally occurring mutation in the COMP gene shows a tendon phenotype.³⁰

Proteoglycans

Proteoglycans consist of a central protein core with O-linked glycosaminoglycan side-chains. They can be divided into two classes — the large proteoglycans (such as aggrecan, the large proteoglycan of cartilage, and the soft tissue equivalent, versican) and the small proteoglycans (such as decorin, biglycan, fibromodulin, lumican, and mimican), all of which are present in equine tendon. The large proteoglycans have numerous highly sulfated glycosaminoglycan side-chains, which hold water and therefore are present where the tendon has to resist compression. The small proteoglycans are closely associated with the collagen fibrils and are believed to regulate collagen fibril diameters. 'Knockout' of the decorin gene in mice resulted in large, irregular-sized collagen fibrils believed to be caused by unregulated lateral fusion and this was associated with weak and fragile skin.³¹ Fibromodulin knockout mice showed paradoxically smaller collagen fibrils but also a compensatory increase in another small proteoglycan, lumican, which binds to the collagen molecule at the same site to fibromodulin,³² demonstrating that many of these small proteoglycans can 'cross-function'. However, tensile testing of the mice's tail tendons showed a significant reduction in tensile strength in the adult fibromodulin-null mice,³³ consistent with the hypothesis that, although another small proteoglycan can do the same job, it does so less well.

Other proteins

There is a wide variety of other proteins in tendon whose functions are only partially elucidated. There is a variety of minor collagens, including type III, predominantly surrounding the fascicles within the endotenon tissue, and type VI, especially in the digital flexor tendons, which also appears to be regulated by mechanical load. Thrombospondin 4, tenascin-C, fibronectin, hyaluronic acid, and small amounts of elastin are also present but their contribution to the function of equine tendon is unclear.

Tendon-specific differences in structure and composition

Considerable debate still surrounds the simple question as to whether all tendons are constructed from the same material and/or have the same basic material properties. Our data would suggest that neither the composition nor the material properties are the same for all tendons, with differences reflecting the functional requirements. Thus, along the length of the digital flexor tendons in all animals weight bearing on a hyperextended metacarpophalangeal joint, where compressive forces are applied to the tendons as they change direction around the palmar aspect of the metacarpophalangeal joint, there is an accumulation of those matrix proteins most suited to resisting that compression. Thus, the tendon has a fibrocartilage-like composition in this region.^{34,35}

Between tendons, there are differences in hydration, collagen content, and some of the non-collagenous proteins. Thus, when comparing the digital flexor tendons, which are weight-bearing tendons, with the digital extensor tendons, which are positional tendons, the flexor tendons have higher hydration and lower collagen content, which is reflected in differences in stiffness. Probably the most dramatic difference is seen in the COMP content during growth.³⁶ Both tendons have similarly low levels at birth but there is a dramatic rise in COMP levels in the superficial digital flexor tendon to 10 times levels at birth, while levels in the common digital extensor tendon do not alter. This probably reflects the different functional requirements between these weight-bearing and positional tendons. Interestingly, similar differences in COMP levels and other minor matrix components, such as type VI collagen, are observed between weight-bearing (e.g. Achilles tendon) and positional (e.g. anterior tibial tendon) tendons in man (Smith & Heinegård, personal communication).

Functional characteristics of tendon

Tendon and ligaments transmit forces to move the equine skeleton, or for support of the distal limb in the case of the digital flexor tendons, or, as ligaments, to maintain joint integrity. At heel strike, the loads rise the quickest in the soft tissue structures primarily sup-

porting the metacarpophalangeal joint — the SDFT and the SL. High-frequency transients are also a feature of the early phase of weight bearing in these tendons. The load in the deep digital flexor tendon is slower to rise, which may help to explain why the SDFT and SL are the most prone to injury.

Load/deformation characteristics

The biomechanical properties of a tendon can be defined *in vitro* by its structural (as an organ) or material (as a tissue) properties. To establish these data, tendons can be recovered from cadavers and pulled to failure in a material testing machine. Anchoring of the tendon ends is problematical as final rupture often occurs at the tendon-clamp interface, which can result in artificially low values. Furthermore, data vary depending on a number of factors including rate of loading. Thus, comparisons between data sets performed in different ways should be made with caution.

In vitro loading experiments generate a load–deformation curve from which the structural properties of ultimate tensile strength (kN) and stiffness (N/mm) can be derived (see Fig. 2.3.7). There are four regions to the curve:

1. The 'toe' region, where there is non-linear stretch to the tendon. This is associated with the elimination of the crimp pattern of the collagen fibrils.
2. Linear deformation — it is this area of the curve from which the stiffness is determined (load divided by deformation for the linear portion of the curve). The mechanism for this elongation is not known but arises from elongation of the collagen fibrils and/or sliding of fibrils relative to one another. Recent work has suggested that interfibrillar (and even interfascicular) deformation is much greater than intrafibrillar/intrafascicular deformation, suggesting that tenocytes survive in a 'strain-protected' environment where they experience considerably lower deformation than that recorded for the whole structure.³⁷
3. Yield region — irreversible lengthening of the tendon occurs at these deformations, possibly arising from covalent cross-link rupture and slippage of collagen fibrils.
4. Rupture, where the stress–strain curve falls quickly to zero as the collagen fibrils sequentially rupture.

Knowing the cross-sectional area of the tendon and its length, the stress (force per unit area) can be plotted

against strain (change in length over original length), from which the material properties of ultimate tensile stress (N/mm²) and Young's modulus of elasticity (E; MPa) can be calculated. As both the cross-sectional area and original length are assumed to be constant, the stress–strain curve has a similar shape to the load–deformation.

Ultimate tensile strength and stress

The ultimate tensile strength for the equine SDFT (rupturing at the mid-metacarpal region) in the horse is approximately 12 kN or 1.2 tonnes. The approximate ultimate tensile stress is 100 MPa for the equine SDFT, which agrees well with previously documented figures for other species (45–125 MPa).^{38,39}

Ultimate tensile strain

Equine flexor tendons can extend 10–12% of their length, and values of up to 20% have been reported¹⁰ before the tendon ruptures. However, the ultimate tensile strain reflects only the final strain before rupture and includes that yield portion of the stress–strain curve that represents irreversible damage to the tendon tissue. In addition, the ultimate tensile strain may not be constant along the length of the SDFT *in vitro*.⁴⁰ Recent work has demonstrated that the normal strains in the digital flexor tendons *in vivo* (in ponies) are in the region of 2–4% at the walk and 4–6% at the trot.⁴¹ Riemersma and colleagues⁴¹ also noted that different results were obtained *in vivo* to *in vitro*, thereby indicating caution in the interpretation of *in vitro* measurements. Other studies have shown that, in the galloping Thoroughbred, strain changes between heel strike and maximum weight bearing can reach 16% in the SDFT.⁴² Such strains — far greater than usually expected in tendons from most other species — may reflect the importance of the digital flexor tendon as an elastic energy store where maximum deformation stores the most energy.

Cyclical loading and pre-conditioning effects

The biomechanical parameters mentioned above can only act as guides to the mechanical properties of tendons as tendon is a viscoelastic tissue.³⁹ Its time-

dependent and history-dependent properties indicate a more complex structure than a simple elastic substance.

Hysteresis

The property known as hysteresis is demonstrated by the difference in the stress–strain relationship when the tendon is loaded compared to when it is unloaded (Fig. 2.3.11). The area between these two curves represents the energy lost during the loading cycle. This is usually about 5% in equine tendons. Much of this energy is lost as heat and is responsible for the rise in temperature within the tendon core associated with repeated loading (as in an exercising horse).⁴³ These temperatures can rise to as high as 46°C, which is potentially damaging to either tendon matrix or tenocytes. However, tenocytes recovered from the center of equine SDFT remain viable when subjected to rises in temperature of this magnitude, whereas those recovered from the periphery of the tendon do not.⁴⁴ This property is also present in fetal tenocytes, which suggests that the tendon has an inherent genetic adaptation to this physical process.

Conditioning

The viscoelastic properties of tendon are demonstrated by movement of the stress–strain curve to the left or

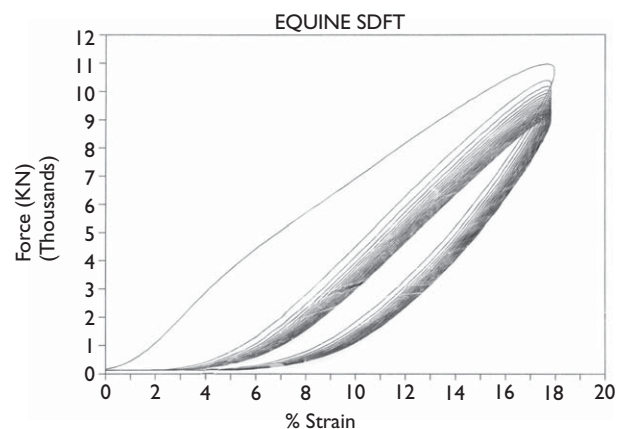


Fig. 2.3.11

The effect of repeated loading and unloading of tendon. The loading and unloading curves are different resulting in a loss of energy (hysteresis). Repeated loading shifts the curve to the right (conditioning).

right (see Fig. 2.3.11). Loading rate has only a minimal effect on tendon biomechanics; a rapid loading rate will move the curve slightly to the left, indicating a stiffer tendon.⁴⁵ Repeated loading, in contrast, results in shifting of the curve to the right (i.e. the tendon becomes less stiff), a process known as conditioning. This change is recoverable but significant resting time is necessary.⁴⁵ This property has, however, been demonstrated in vitro and may not reflect the normal behavior in vivo.

Contribution of tendon mechanics to the energetics of locomotion

With tendon loading under weight bearing being essentially a passive process, energy is stored in the extension of the tendon. As the energy stored in the tendon is represented by the area under the stress–strain curve, for this system to operate with maximum efficiency, the tendon must stretch as much as possible. As this tendon will rupture with in vitro testing at between 12 and 20%, the SDFT in vivo strain levels of up to 16% at the gallop⁴² demonstrate that the high efficiency comes at a cost — there is little tolerance in the system and the tendon is prone to overstrain injury. Consequently, any small deterioration in the mechanical properties would have a significant effect on the risk of tendon injury.

The tendon returns energy with an efficiency of approximately 93%,⁴⁶ which provides considerable energy-saving for the horse. Predicted efficiencies of locomotion in the horse at different gaits calculated from the energy expenditure required for limb and trunk movement and the movement of the horse, and the energy production by the muscles (which have an efficiency of approximately 30%) show an efficiency of greater than 100% at the gallop. This discrepancy is due to the energy-storage capacities of the locomotor soft tissues, in particular the SDFT and SL. Thus, these structures are critical to the optimum efficiency of equine locomotion.

Age and exercise effects on tendon pathophysiology

With preceding tendon degeneration proposed as an important factor in the initiation of equine superficial digital flexor tendinopathy, investigations have con-

centrated on changes occurring with the tendon matrix associated with aging and exercise. The importance of these factors on tendon physiology has been supported by epidemiologic studies in both horses (superficial digital flexor tendinopathy)^{2,4} and man (Achilles tendinopathy).^{6–8}

A combination of in vivo and post-mortem studies on ‘normal’ horses, both domesticated (trained) and feral (untrained), and analyses of tendons from a number of experimental exercise studies has shone new light on the influence of both exercise and aging on equine tendon. These studies have investigated mechanical, morphological (ultrasonographic and histologic), ultrastructural, compositional, and metabolic changes in association with aging and exercise in equine tendon. The effects of these two factors have been found to be different between immature (growing) and mature (adult) tendon. The age at which equine digital flexor tendon matures is estimated at approximately 2 years of age from these studies.

Mature (adult) tendon

Mechanical changes

There is a large variation in mechanical properties between individuals — more than twofold for ultimate tensile strength¹⁰ — so that alterations in both structural and material properties are difficult to determine. In vitro testing from a large number of horses has demonstrated a decrease in ultimate tensile stress with age, although this was not significant.⁴⁷ In further support of this hypothesis, human studies in vivo have demonstrated a decrease in stiffness in the Achilles tendon with age.⁴⁸ As this was an in vivo study, no ultimate tensile strength measurements are possible, although reduced stiffness is often associated with reduced strength in collagenous soft tissues.

The effect of exercise on the mechanical properties of tendon and ligament is variable, although much of this variation may be due to different ages and species used.

Morphological changes

The crimp pattern of equine SDFT was found to reduce in both angle and length with age.^{49,50} Furthermore, the imposition of exercise resulted in an accelerated

loss of this crimp in a heterogeneous manner — the central region of the tendon was disproportionately affected.⁵¹ This may help to explain the occurrence of central ‘core’ lesions seen clinically as the central fascicles, having less crimp, straighten first and are therefore loaded preferentially, becoming the first ones to rupture.⁴⁹

Other studies have assessed the change in fascicle pattern with aging and training.⁵² Here, a significant reduction in the number of fascicles with a thickening of the interfascicular (endotenon) tissue was found associated with aging. This correlates well with the location of transforming growth factor-beta (TGF- β) in maturity (see ‘Metabolic changes’ below) and is consistent with this part of the tendon being the most labile portion of the tendon.

The effect of exercise on the cross-sectional area of tendon has shown inconsistent results. Ultrasonographic and gross cross-sectional measurements during experimental treadmill exercise in young adult horses failed to show any significant change with exercise over and above that caused by growth in digital flexor tendons.⁵³ In contrast, the common digital extensor tendon did show a significant increase with exercise, confirming similar findings in exercise studies on mini-pigs.⁵⁴ Another study, following cross-sectional area changes with the onset of training, showed a significant increase.⁵⁵ However, two of the seven horses followed developed evidence of clinical tendon injury and so it is difficult to relate such increases in cross-sectional area to either adaptation or injury.

Ultrastructural changes

Collagen fibril diameters, calculated using transmission electron microscopy, in the adult SDFT in horses show a biphasic distribution, with a large number of small (40 nm) fibrils and a low number, but broader range, of large fibrils, while very old tendons have a more unimodal distribution characteristic of fetal and newborn tendon.²⁴ The bimodal distribution was unaffected by a short-term (4.5 months) experimental treadmill exercise program in ~2-year-old horses but there was a shift to smaller-diameter collagen fibrils in the longer-term study (18 months) where the horses were over 3 years of age⁵⁶ (Fig. 2.3.12). This effect was believed to be due to the disruption of large fibrils rather than the formation of new ones as the collagen content and ‘age’ (as determined by non-enzymatic glycation) were unaltered.

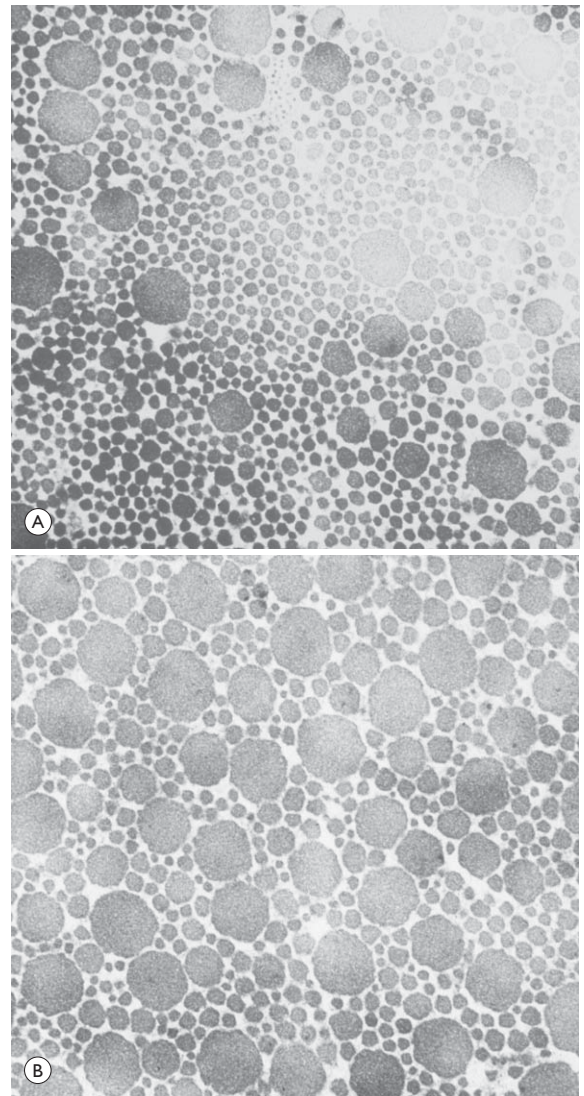


Fig. 2.3.12 Transmission electron microscopy of superficial digital flexor tendon in 3-year-old Thoroughbreds after 18 months of treadmill exercise (A), compared with controls that received only walking exercise (B). Exercise has induced a greater number of small-diameter fibrils. (Courtesy of the Equine Veterinary Journal, reproduced with permission.)

Compositional changes

Collagen content varies little with age and exercise but the non-collagenous component of tendon is much more labile. Analysis of tendons from a wide range of

ages, together with those from experimental exercise studies, has shown a decrease in glycosaminoglycan (GAG) levels with age and exercise, where both act synergistically.⁵⁷ This is in contrast to the central discolored regions seen as an occasional coincidental finding at post mortem, which contain increased GAG levels suggestive of scar tissue formation⁵⁸ (see Fig. 2.3.3). GAGs are part of proteoglycan molecules but vary in amount between different proteoglycan species. Thus, the small proteoglycans of tensile tendon contain only one or two side-chains of GAGs while the large proteoglycans, more characteristic of areas of tendon under compression (e.g. where the digital flexor tendons change direction at the level of the metacarpophalangeal joints), contain large numbers of GAG side-chains. Thus, changes in GAG levels within tendon probably reflect more changes in the large, rather than small, proteoglycan species.

Another non-collagenous protein, COMP, which is abundant within the digital flexor tendons of horses, shows marked changes with age and exercise. Levels are low at birth but rapidly increase with tendon growth during the first 2 years of life. Once maturity is reached, which is at approximately 2 years in tendon, levels rapidly decline but only within the tensile (metacarpal) regions of the digital flexor tendons.²⁷ The superimposition of exercise at this time results in a significant increase in the loss of COMP from this area of the tendon.¹² In contrast, levels of COMP within the low-loaded tendons, such as the common digital extensor tendon, are also low at birth but do not alter significantly with growth and aging,³⁶ although exercise did alter levels in immature tendon (see below).

Metabolic changes

Recent studies investigating the distribution of the growth factor TGF- β , a potent anabolic growth factor and stimulus for COMP synthesis *in vitro*, have demonstrated reduction in the presence of the three isoforms of TGF- β after skeletal maturity, although TGF- β 1, believed to be most associated with scar tissue formation in wound healing, remains prominent within the interfascicular (endotenon) tissue where age-associated thickening occurs.⁵⁹

These findings have resulted in the hypothesis that the tensile regions of the digital flexor tendons of the horse lose their ability to adapt to exercise after skeletal maturity and both age and exercise provoke degeneration in the tendon matrix, characterized by a loss in non-collagenous proteins. In support of this hypoth-

esis, analysis of matrix gene expression in bovine digital flexor tendons has shown prominent matrix gene expression at birth and during growth but a complete absence of gene expression in the tensile (but not compressed) regions.⁶⁰ Interestingly, COMP levels are also maintained in the compressed regions of equine digital flexor tendons and this area is frequently spared clinical injury. The mechanism for this failure of tenocytes to produce tendon matrix in the adult is unclear, but might involve either an absence of appropriate growth factor stimulus or cellular senescence. Certainly, investigations into the synthetic response by equine tenocytes to mechanical load (biaxial stretch) and growth factors (TGF- β) *in vitro* have demonstrated little response to mechanical load alone.¹³ TGF- β has a major effect on protein synthesis and, when combined with mechanical load, is synergistic. However, tenocytes recovered from aged flexor tendons do demonstrate a small, but significant, reduced response to load and TGF- β , suggesting that the failure of an adaptive response in adult tendon is potentially due to a combination of the absence of growth factors and, to a small degree, cellular 'senescence'. Interestingly, this age effect is not apparent in tenocytes recovered from digital extensor tendons.

Mechanisms of strain-induced degeneration

The close association between age and exercise suggests that number of loading cycles is important. It is logical to presume that the highest loading rates are likely to be the most damaging, so the amount of time spent at the fastest gaits (canter and gallop), where strains can reach 16% with an initial strain rate of up to 200%/s, are likely to be the most contributory for degeneration. To combat these deleterious effects of exercise, it has been proposed that the muscle of the SDFT primarily acts to fix the origin of the tendon and dampen the potentially harmful high frequency vibrations.¹⁷

The actual mechanism for the degeneration of the tendon is currently unknown, although there are several possibilities. These mechanisms can be either physical or metabolic processes. The physical energy imparted to the tendon under weight-bearing load can produce direct damage to the matrix by disrupting cross-links or actual matrix proteins. An indirect physical effect of weight bearing is via the energy lost through hysteresis. This results in a temperature rise within the center of the tendon.⁶¹ While the tenocytes in the superficial digital flexor tendon have been

shown to be resistant to these temperature rises,⁴⁴ this temperature could still be damaging to matrix proteins. Loading cycles can induce cellular activity with potential release of proteolytic enzymes.⁶² Furthermore, cleaved matrix proteins, generated either from direct physical forces or from enzymatic cleavage, can also provoke further matrix degradation.⁶³ Further work is necessary to elucidate the mechanisms of soft tissue degeneration so that preventative strategies can be developed.

Immature (growing) tendon

Although there appeared to be a failure in adaptive response to exercise in mature digital flexor tendon in the studies outlined above, is this also the case in immature tendon? Two recent studies have addressed this question — the first involving three different exercise regimes (box rest, box rest with enforced exercise (training), and pasture exercise) in Warmblood foals from 6 weeks to 5 months of age,⁶⁴ and the second, an increasing amount of treadmill exercise in Thoroughbred foals from 6 weeks to 15 months of age.⁶⁵

Mechanical changes

In the Warmblood study, foals kept at pasture had significantly stronger tendons structurally at 5 months of age than the other two groups, largely due to the development of a larger cross-sectional area (see below), as the ultimate tensile stress at rupture (material property) was not different between groups. By 11 months of age, after all three groups had received a 6-month period of low-level exercise, the differences between groups were largely no longer present.

Morphological changes

The recent study by Kasashima and colleagues⁶⁴ has documented a significant rise in the rate of increase in cross-sectional area of the superficial digital flexor tendon with treadmill exercise administered for only a small period each day in addition to pasture exercise. Interestingly, the variation in tendon cross-sectional area between limbs increased in both exercise and control groups towards the end of the study. In the study on Warmblood foals, the cross-sectional area was significantly larger at 5 months of age in the foals kept on pasture in comparison to those maintained on box rest or box rest with small bouts of enforced

intense exercise. These differences were no longer significant at 11 months after all three groups had received a similar level of low-level exercise for 6 months.

Ultrastructural changes

Fetal and newborn equine SDFT has a uniform fibril size of moderate diameter (i.e. not universally small).^{24,66} During growth, a bimodal distribution of fibril sizes, with the largest number of small-diameter (~40 nm) fibrils and lower numbers of large fibrils (>200 nm in diameter), becomes apparent within the first year of life.⁶⁶ These small fibrils may represent either new collagen fibrils waiting to be incorporated into larger fibrils, different collagen (type III has universally smaller fibril diameters than type I), or the disruption of larger-diameter fibrils. This change in fibril diameter distribution appears to be influenced by loading and exercise, as different exercise regimes given to foals altered the time at which the small fibrils appeared.⁶⁶ Pasture exercise resulted in the most rapid appearance, with a predominance of small fibrils present by 5 months of age. Foals maintained in a box had a delayed onset of this 'adult' phenotype.

In comparison, treadmill exercise given to foals from 6 weeks to 15 months of age failed to alter the fibril diameter distribution between controls and exercised foals (Kasashima, personal communication), although these foals also had access to pasture exercise, which may have been sufficient to induce the bimodal distribution of fibrils.

Compositional changes

COMP levels have been found to be exquisitely sensitive to loading *in vivo* early in life. COMP failed to accumulate in the digital flexor tendons of an 11-week-old foal that had been non-weight bearing on one limb for 6 weeks. This effect was not marked if the period of non-weight bearing occurred after COMP had accumulated within the digital flexor tendons.²⁷

In 5-month-old foals given pasture exercise, box rest, or box rest with enforced exercise (training) from 7 days of age there were demonstrated changes in molecular composition with respect to exercise.⁶⁵ Both COMP and polysulfated glycosaminoglycan (PSGAG) levels were lowest in the training group, suggesting that this exercise regimen was detrimental to tendon development. After normalizing the exercise across all groups for a further 6 months, there was only limited

ability for the tendons to recover, although there were changes associated with growth, in particular increases in hyaluronic acid and COMP. Thus, early exercise is potentially the most important determinant of tendon development.

In contrast, in the exercise study performed by Kasashima and colleagues,⁶⁴ COMP levels were not altered in the SDFT, although significant rises were induced in the CDET. This further supports earlier work in miniature swine by Woo and colleagues,⁶⁶ who demonstrated a significant change in tissue properties for digital extensor tendons and ligaments but not digital flexor tendons. It may be that the load 'history' over the 15 months of exercise was not sufficiently different between the two groups to induce a significant change in the digital flexor tendons.

Metabolic changes

TGF- β isoform expression was found both within and between fascicles in young tendon, mainly pericellular in location.⁵⁹ Interestingly, polymerase chain reaction (PCR) analysis of TGF- β gene expression showed no growth factor expression after birth, except when the tendon was injured, suggesting that the tendon TGF- β stores are fixed and limited at birth.

These investigations suggest that tendon is able to respond to exercise during growth. However, this response appears to be dependent on the exercise regimes. In the study using Warmblood foals,⁶⁴ pasture (constant) exercise appeared to produce a better-quality tendon than that resulting from either box rest (limited, low-level exercise) or enforced exercise with box rest (limited but high-level exercise). Some, but not complete, recovery appeared to be possible between 5 and 11 months when the animals were allowed low-level free exercise. This study also confirmed that the 'window of opportunity' for tendon adaptation appeared to be early in the life of the animal.

The minimal changes induced by additional, albeit small, amounts of treadmill exercise suggested that the natural exercise level at pasture may be optimal for tendon development. Certainly, the gamboling activities of foals at pasture would appear to be ideally suited for high strain rate controlled loading of the digital flexor tendons (Fig. 2.3.13). It may be that both time and intensity 'windows of opportunity' exist, above or past which further augmentation of tendon properties can not be achieved.



Fig. 2.3.13

Gamboling activity of foals at pasture may be ideally suited to the development of tendons. (Courtesy of Dr Yoshinori Kasashima, Japan Racing Association.)

Tendon injury and repair

Gross damage and mechanical failure

With the advent of clinical injury, the extent of the damage to the matrix varies from disruption of fibrillar cross-links (covalent and electrostatic), to individual fiber ruptures and ultimately failure of the entire tendon. This damage can be focal or generalized and one of the more common manifestations of superficial digital flexor tendinitis is a centrally located region of injury (so-called 'core' lesion seen ultrasonographically; Fig 2.3.14), usually with the most severe level being just below the mid-metacarpal region but also extending throughout most of the length of the metacarpal extrasynovial tendon. Regions of the SDFT enclosed within a tendon sheath (carpal sheath proximally; digital sheath distally) are usually much less severely affected, although this can be relatively more common when the metacarpal region has been previously injured.

Desmitis of the accessory ligament of the DDFT can occur as an isolated injury or in conjunction with superficial digital flexor tendinitis. Its pathogenesis is therefore related more to the SDFT than the DDFT to which it attaches. While ponies rarely suffer strain-induced tendinopathy, they do have a relatively high incidence of desmitis of the accessory ligament of the DDFT.

The SL can fail at any site along its length, although certain areas are more common in horses used in dif-

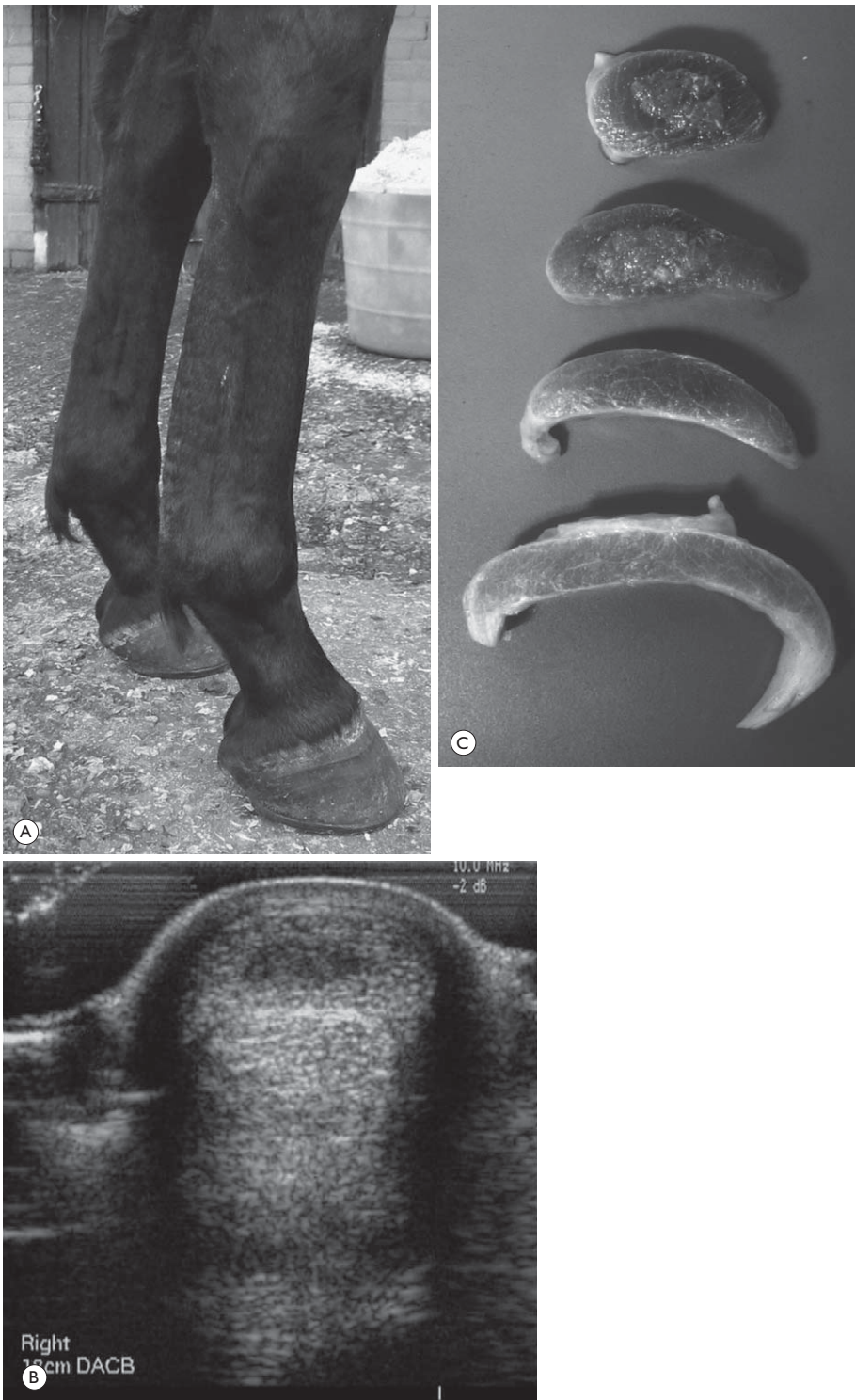


Fig. 2.3.14

(A) Clinical, (B) ultrasonographic, and (C) post-mortem appearance of the 'core' lesion — a common manifestation of superficial digital flexor tendinitis.

ferent disciplines. Thus, race horses tend to suffer lesions of the body (and branches) of the SL, while sports horses more frequently have pathology centered within the proximal or branch regions of the ligament.

In contrast to the SDFT and SL, the deep digital flexor tendon (DDFT) is most frequently injured within the digital sheath. It is not known whether these injuries have a preceding phase of tendon degeneration but many are potentially due to single loading cycles which induce overstrain damage. Consequently, bilateral pathology is rarer. Two manifestations are seen clinically. The first arises within the substance of the tendon (although it may extend to the borders of the tendon), which is similar to other clinical manifestations of tendinitis. The second arises at the medial or lateral borders of the tendon, usually in the region of the metacarpo-/metatarsophalangeal joint, with no, or limited, involvement of the internal substance of the tendon. These tendon 'tears' are thought to arise from 'bursting' pressures within the tendon when the metacarpo-/metatarsophalangeal joint is overextended.

Other tendons can suffer from strain-induced injury, although much less commonly than that affecting the palmar soft tissue structures of the metacarpus. Ligament injuries tend to occur when the joint they span is loaded inappropriately to result in a degree of subluxation.

Repair processes in tendon

Once the tendon suffers clinical injury with disruption of the tendon matrix, there is intratendinous hemorrhage initially, usually followed rapidly by a pronounced inflammatory reaction. This inflammatory reaction results in an increase in blood flow, the development of edema, infiltration of neutrophils, macrophages and monocytes, and the release of proteolytic enzymes. While this is the earliest stage of repair, designed to remove damaged tendon tissue, the response is usually excessive, causing further damage to the tendon. This inflammatory phase is usually short-lived but, within a few days, the reparative phase of repair begins. This results in a pronounced angiogenic response and the synthesis of scar tissue. This tissue has a different composition to tendon, having a higher ratio of collagen types III/I (~50%, cf. 10% for normal tendon; Birch, personal communication), higher levels of glycosaminoglycans, and much lower levels of COMP.²⁷

The reparative phase of tendon healing merges with the remodeling phase when there is a gradual but incomplete transformation of collagen type III to I as the scar tissue matures.⁶⁷ The new collagen fibrils become thicker and cross-linked. Even mature scar tissue tends to be less stiff as a material than tendon, but because large amounts are formed, the scarred tendon often becomes stiffer as a structure than the original tendon.¹⁵

Diagnosis

Diagnosis of tendon injury is usually based on history (frequently a preceding bout of intense exercise) and the development of the signs of inflammation (pain, heat, swelling, and lameness) over the affected structure (see Fig. 2.3.14). Lameness may not always be present and tends to be related to the degree of inflammation rather than the degree of damage. In many cases, however, the onset of clinical tendinitis is associated with severe lameness.

The posture of the limb may be altered depending on the structure damaged and the severity of the injury. In the case of severe superficial digital flexor tendinitis, the resting metacarpophalangeal joint angle may be normal because of the action of the other supporters of this joint (SL and DDFT). However, when loading on the limb increases (e.g. when the contralateral limb is raised), the affected limb shows greater than normal overextension of the metacarpophalangeal joint. Severe damage to the SL will have greater effect on metacarpophalangeal joint extension.

For more subtle cases, careful palpation of the tendons in the limb should be made both with the limb weight bearing and raised. Careful attention should be made to pain response, subtle enlargement, and consistency of the structure (soft after recent injury; firm after healing). The horse must be relaxed so that muscle activity does not tense the tendons and make them appear artificially firm. Careful visual assessment of 'bowing' of the palmar contour of the metacarpal region can help to identify subtle superficial digital flexor tendinitis.

Clinical examination, however, may not detect the most subtle injuries and assessment of the severity is limited by clinical examination alone. As prognosis is most dependent on the severity of the initial injury, it is prudent to evaluate the damaged area ultrasonographically and this is best carried out 4–7 days after the onset of the injury as many lesions expand during the initial few days. Modern ultrasound machines

with a 7.5–10 MHz linear transducer produce excellent-quality images of the flexor tendons and SL. While the metacarpal region can be evaluated ultrasonographically without clipping the hair, it is recommended to prepare the limb by clipping and washing (with a surgical scrub and spirit) to give the best-quality images. The horse should be standing square and both transverse and longitudinal images obtained in a methodical fashion throughout the length of the region containing the injured tendon. For the metacarpal region, the area is divided up into seven levels or zones, each with characteristic anatomy. Alternatively, the distance between the transducer and the accessory carpal bone can be recorded. The palmar soft tissue structures of the metacarpus can be evaluated satisfactorily from the palmar aspect of the limb, except for the SL branches which should also be evaluated from the medial and lateral aspects of the limb. Both limbs should always be examined as many cases of strain-induced tendon injury have bilateral components but with one limb more severely affected than the other.

Acute tendon pathology is manifest ultrasonographically by enlargement, hypoechogenicity (focal, e.g. 'core' lesion — see Fig. 2.3.14; or generalized), reduced striated pattern in the longitudinal images, and changes in shape, margin, or position. Chronic tendinopathy is associated with variable enlargement and echogenicity (often heterogeneous), and a reduced irregular striated pattern indicative of fibrosis.

Markers of tendon injury

When a tendon is injured, proteins are released from the tendon into either a surrounding synovial fluid (for intrathecal injuries) and/or the blood (for extra-thecal injuries). It is potentially possible to detect these released proteins in either tendon sheath synovial fluid or blood, which could then be used as a molecular marker of tendon disease.

The development of a specific assay for a molecular marker of tendon injury relies on one of two different approaches. One alternative is to identify a protein which is specific for tendon tissue and released into the bloodstream following injury. Studies on the molecular composition of tendon using 2D gel electrophoresis (Smith & Heinegård, personal communication) have demonstrated that there are many similarities between the proteins present in cartilage and tendon and few, if any, specific for tendon tissue, making this approach less viable at present.

The second alternative is to identify a protein that is not specific to tendon but whose distribution is restricted and/or whose fragmentation with injury is specific for tendon injury. One such protein is COMP, which has a restricted distribution to tendon, ligament, cartilage, intervertebral disk, and meniscus. Furthermore, COMP is particularly abundant in young mid-metacarpal SDFT, the area most prone to injury. After skeletal maturity, the natural decrease in COMP levels within the metacarpal region of the SDFT can be accelerated by exercise.¹² These findings suggest that it might be a useful indicator of tendon damage if, once it is released from the metacarpal region of the SDFT, it gains access to the bloodstream and can subsequently be assayed. COMP is not significantly absorbed by the peripheral lymph nodes (cf. proteoglycan fragments; Frazer et al, unpublished data) and COMP fragments are found in the serum in humans.⁶⁸ While studies in man have demonstrated that COMP has potential usefulness as a marker of joint disease,⁶⁹ assay of the total amount of COMP in serum showed no significant alterations in COMP levels with tendon disease,⁶⁸ although there were significant increases associated with the commencement of training (Smith & Bathe, unpublished data). This assay quantifies COMP using a polyclonal antiserum that recognizes a number of epitopes on the COMP molecule. There is a normal significant background level of COMP in the serum (1–2 mg/mL), possibly representing the normal turnover of COMP from all tissues containing the protein. Certainly there are considerably higher levels of total COMP in the serum of growing horses compared to adults,⁶⁸ when COMP is being accumulated in the tissues. In addition, damage to cartilage in joint disease also contributes to the 'pool' of COMP in the serum and is therefore responsible for a reduction in the sensitivity of the assay for tendon disease itself.

However, COMP levels in digital sheath synovial fluid in horses with intrasynovial tendon injury demonstrated significant rises. We know from previous studies that there is <1/100 the level of COMP in the digital sheath capsule,²⁷ so that rises observed in the digital sheath synovial fluid (approximately four times normal levels) suggest that COMP is lost from the intrasynovial tendon rather than the digital sheath. The COMP released within the digital sheath synovial fluid is composed of fragments which have been identified on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after partial purification with ion exchange chromatography. This fragmentation pattern is different from that described for human

joint diseases,^{69,70} although may not be in the horse. These fragments represent ideal candidates for markers of tendon disease. Assay of these fragments, not normally present, would potentially enable a very sensitive assay to be developed.

In addition to proteins released after injury being useful for the detection of tendon damage, the healing process can also potentially be monitored using markers of protein synthesis. While serological markers of collagen synthesis (e.g. propeptide of collagen type I; PICP) were thought to be relatively specific for bone remodeling, recent studies have demonstrated significant rises of PICP following tendon injury.⁷¹ Further work is necessary to determine if this molecular marker will be useful for the monitoring of tendon repair.

Review of current treatment strategies

Over the years, many treatment modalities have been tried, with most showing equivocal or even deleterious effects. From our knowledge of the phases of tendon healing, the following have at least a rationale for treating tendinitis.⁷²

Acute (inflammatory) phase

Physical therapy (rest, application of cold, and compression with bandaging) is the most important aspect of early management where the goal is to minimize inflammation and limit the action of proteolytic enzymes that continue to destroy tendon tissue. Pharmacologic interventions include short-acting steroids within the first 24–48 h (later application can inhibit the second phase of fibroplasia) and the use of polysulfated glycosaminoglycans, which have been shown to inhibit prostaglandin E₂ production *in vitro*.⁷³ The intralesional use of steroids should be avoided, as this has been associated, at least with depot preparations, with intratendinous calcification. Surgical treatment at this stage includes percutaneous tendon splitting, which has been shown to accelerate the resolution of the 'core' lesion seen ultrasonographically.⁷⁴ This can be done with a scalpel or, less invasively, performed with needle puncture, when it can be combined with intratendinous polysulfated glycosaminoglycan therapy. Desmotomy of the accessory ligament of the SDFT, often performed concurrently with percutaneous tendon splitting early on in the

disease process, is suggested to reduce peak strains on the SDFT by bringing the superficial digital flexor muscle into play.⁷⁵ However, although initial data suggested a beneficial effect,⁷⁶ this has not been confirmed in other studies and has been suggested to contribute to a higher incidence of suspensory desmitis after its use.⁷⁷

Subacute (fibroblastic) phase

Early and progressive mobilization and regular ultrasonographic monitoring aim to improve the quality of the forming scar tissue — the goal of this phase. If the cross-sectional area of the healing tendon increases by more than 10%, the exercise level should be reduced. The quality of the longitudinal fiber pattern when the animal returns to full work has been linked with the overall prognosis.⁷⁸ In an attempt to improve the quality of the scar tissue, β -aminopropionitrile fumarate (BAPTENTM; no longer marketed for the treatment of tendon injuries) has been injected intratendinously 30–90 days after injury.⁷⁸ This drug inhibits lysyl oxidase, the enzyme that cross-links collagen molecules. In preventing the formation of cross-links, it is believed that collagen fibers will form with better longitudinal alignment under the stimulus of controlled exercise. When the drug wears off, cross-linking occurs and the scar increases in strength. Clinical trials in the USA indicated a benefit in the more severe cases.

Other drugs, such as sodium hyaluronate, have been used both intratendinously and peritendinously but studies have shown equivocal results. Some benefit in reducing adhesion formation in intrasheath tendon injuries has been demonstrated experimentally.

Newer treatments aimed at regenerating rather than repairing tendon represent the best hope for the future. Anabolic growth factors, such as insulin-like growth factor (IGF)-1,⁷⁹ recombinant equine growth hormone,⁸⁰ and TGF- β , have been tried empirically or in a collagenase model of tendon injury, but not comprehensively evaluated in the clinical situation, making interpretation of the benefits of these agents difficult. An important factor in assessing these new treatments is that they do actually generate tendon-like tissue rather than just increasing the amount of scar tissue produced, which will still compromise the outcome. One of the most exciting new developments is the use of mesenchymal stem cells (recovered from bone marrow),^{81,82} although much work is still necessary to determine their effectiveness.

Chronic (remodeling) phase

A controlled ascending exercise program with regular ultrasonographic examinations encourages the further optimization of the scar tissue. In addition, ultrasound can enable the detection of early signs of reinjury to minimize the risk of catastrophic reinjury. Other methods aimed at preventing reinjury include desmotomy of the accessory ligament of the SDFT (see 'Acute phase' treatments) and the use of boots capable of providing significant support to the metacarpophalangeal joint. Traditional bandages fail to provide sufficient support under weight-bearing load, while novel designed boots have been shown to do so and potentially may help to prevent reinjury⁸³ (Fig. 2.3.15).

Specific therapies

Some tendon and ligament injuries have specific therapies in addition to those outlined above. DDFT tears within the digital sheath and SL tears into the metacarpo-/metatarsophalangeal joint are best treated by tenoscopic/arthroscopic debridement. The outcome of hindlimb, but not forelimb, proximal suspensory desmitis appears to be improved (from 13% to 43%) by the use of extracorporeal shock wave therapy.^{84,85} For those cases of hindlimb proximal suspensory desmitis also failing to respond to shock wave therapy, surgical neurectomy of the lateral plantar nerve combined with transection of the fascia overlying the proximal suspensory ligament provides an additional rational



Fig. 2.3.15

(A, B) Dalmar support boot capable of being used in the exercising horse and able to provide significant support to the metacarpophalangeal joint, unlike traditional bandages. This may provide a method of minimizing reinjury after rehabilitation. (Courtesy of the Equine Veterinary Journal, reproduced with permission.)

management technique. Both these latter two treatments are consistent with the hypothesis that hindlimb proximal suspensory desmitis is an example of a compartmental syndrome, unlike the forelimb (which carries a considerably better prognosis with conservative management alone).

Current concepts in prevention of tendinitis in the equine athlete

Strategies for the prevention of equine tendinitis

With the limitation of our ability with current treatments to return a horse with tendinitis to full work without danger of reinjury, prevention of the injury has to be considered as the most appropriate strategy. From our understanding of tendon physiology described in the preceding sections, four broad approaches for prevention can be considered.

Maximize the quality of the tendon prior to skeletal maturity

There is a large variation in the strength of the SDFT in a population of horses. Some of this variation may be due to specific genes. Either breeding out, or identifying horses with, those genetic variants associated with a genetic susceptibility to tendinitis would potentially lower the incidence of tendon injury. However, while the concept is simple, its achievement is considerably more difficult.

Skeletal tissues are much more able to adapt to the loads placed on them when they are immature and growing. This is certainly true for bone and muscle and we believe it to also be the case in tendon. Thus, carefully tailored exercise regimes during growth (0–2 years of age) would potentially improve the ‘quality’ of the tendon and minimize the effects of degeneration induced by training and racing after skeletal maturity (approximately 2 years of age in the horse) (Fig. 2.3.16). These exercise regimes must be within the ‘windows of opportunity’ of the right time and the right level. Growing tendon is also more susceptible to injury so these ‘conditioning’ programs have to induce adaptation suitable for subsequent racing without causing injury. However, the optimal property of

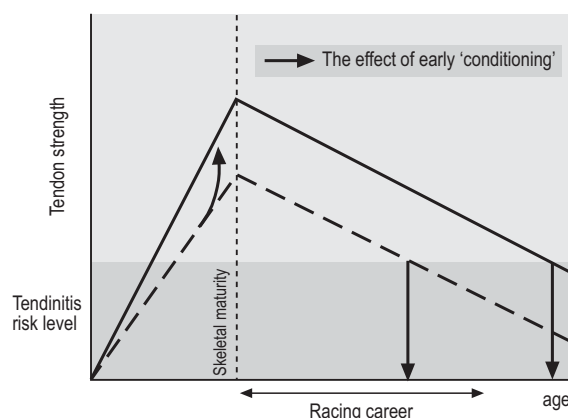


Fig. 2.3.16

Hypothetical response of tendons to the early introduction of exercise. The two lines refer to two different horses — one that develops poor-quality tendon during growth (dashed line) and one that develops good-quality tendon (solid line). Both accumulate damage within the tendon associated with post-skeletal exercise and aging, which inevitably and progressively weakens the tendon. However, in the former, this weakening predisposes the individual to clinical tendinitis within its racing career, whereas the latter is able to survive its racing career without suffering such injury. The imposition of ‘appropriate’ conditioning exercise during growth is hypothesized to improve the quality of the growing tendon, resulting in an individual more resistant to subsequent injury. (Courtesy of the Journal of Comparative Biochemistry and Physiology, reproduced with permission.)

equine tendon still needs to be determined. Probably the most important characteristic would be fatigue resistance and, for any exercise regime in immature animals to be effective, it must demonstrate a reduction of tendon injuries within the subsequent athletic careers. The answers to these questions are not known but studies are underway to assess the effects of early exercise on musculoskeletal development.

Reduce the degeneration after skeletal maturity

Studies are turning towards investigating the mechanisms of aging in soft tissues, which may give therapeutic options for intervening in the aging process. At present, the only advice that can be given in this area is to avoid training solely aimed at the tendon in the adult horse. This will serve only to advance the rate of degeneration, which has to be

considered an inevitable consequence of athleticism. Some forms of exercise may be more provocative of degeneration than others, but, at present, these are not known. However, high loading (i.e. fast speeds) is likely to be the most damaging.

Reduce the risk factors for tendinitis

The initiation of clinical tendinitis is provoked by sudden peak forces that overcome the strength of the (degenerated) tendon. This can obviously occur at any time, including out at pasture, but it is obviously most likely when the horse is loading its tendon maximally. This occurs when the horse is running fastest (hence the best horses are potentially more prone to tendon injury). Ground surface (which affects the horse's speed), fatigue (e.g. after longer races or in unfit horses), jumping, shoeing, and weight are all examples of factors that can increase the peak loading on the tendon and hence are risk factors for tendon injury. Some of these are rectifiable but others are a consequence of racing and not easily altered.

Early detection

This is not really a prevention strategy, as, by definition, a degree of tendon injury will already have occurred. However, if tendon injury can be detected very early, it is possible to prevent progression to more severe disease. Obviously, careful clinical inspection is vital in this area, but this is rather insensitive.

Ultrasonography has been the mainstay of tendon imaging and has advanced our knowledge and capabilities for management considerably. Ultrasonographic technology is still advancing and new machines have even better resolution. However, they are still relatively insensitive for predicting injury and

the ideal time for a return to full work in the chronic stages. Furthermore, it is time-consuming and (frequently) requires the limbs to be clipped.

Future techniques in this area may rely on molecular markers of tendon injury. Fragments of proteins within the tendon, which are released with injury, can potentially be assayed for in the blood, which would give a practical test that would provide useful diagnostic, management, and prognostic information.

Recommendations for training of immature and mature animals

Pasture exercise early in life is essential for the development of digital flexor tendon that is likely to be most resistant to injury during adulthood. Additional imposed exercise may be able to augment this effect but the tendon is also more susceptible to injury at this age and so exercise levels have to be chosen with care. In addition, what about the other skeletal tissues? How do they respond to these exercise regimes? It would be pointless to develop a training regime beneficial for tendon but harmful to other tissues, such as bone and joints. However, different skeletal tissues mature at different times, thus potentially allowing different time 'windows of opportunity' for different tissues. We would suggest that tendon, ligament, and possibly cartilage are most responsive in the first year of life, while bone is more responsive at the yearling stage.

After skeletal maturity, training will have no effect on tendon adaptation and therefore training should be directed at muscular, respiratory, and cardiovascular fitness rather than the tendon.

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Joint physiology: responses to exercise and training

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Joint physiology and structure

The joint is an organ composed of synovium, articular cartilage, and subchondral bone, with a local blood supply, innervation, and fluid exchanges that function to maintain health and produce locomotion.¹ The joints are uniquely designed for rotary or hinge-like movement to permit limb and body movement. Joint tissues adapt to the magnitude and frequency of applied load which naturally occurs with exercise. Training (a forced exercise regimen) is designed to promote the adaptation of the joint structures and physiologic responses to permit high performance without joint compromise. The purpose of this chapter is to provide a comprehensive understanding of the consequences and complexities of physiologic and pathophysiologic changes that occur in the joint during training and exercise.

Basic anatomy and physiology

Tissues of the joint cavity have a specialized composition and three-dimensional anatomy which ultimately relate them to their specific biomechanical function. As such, the articular cartilage, whose ultimate function is to absorb and transfer loads, is a composite of collagen which provides tensile strength and highly charged proteoglycan subunits which provide compressive stiffness. In order to maintain cartilage composition and function, a slow but steady turnover of its components occurs, the rate of which is dependent on age, mechanical load, and joint environment.^{2–5} The building materials necessary for this turnover are provided through blood flow and exchanges from the synovial membrane and joint capsule, as well as through ultrafiltration and formation of synovial fluid, which provides the ultimate medium for these

exchanges to occur.⁶ The supply of nutrients to the avascular but metabolically active articular cartilage is provided by exchanges through the synovial microcirculation and transport in synovial fluid.

The synovial membrane is designed to provide a pathway for fluid exchanges, as well as a blood supply, and add to the composition of synovial fluid through hyaluronan synthesis. The synovial membrane is composed of an intima (also referred to as synovium), consisting of a luminal layer 1–5 cells deep, a capillary plexus which lies 6–11 mm beneath the intimal surface, a deeper network of lymph vessels, and a subintima (or subsynovium) composed of adipose, areolar, or fibrous tissue.⁶ The intima is designed to favor exchanges between capillaries and the joint cavity; it lacks a basement membrane, intercellular gaps are present, and intimal capillaries are fenestrated towards the joint cavity.⁶ Furthermore, the synovial intima adopts a three-dimensional architecture which is dependent on its biomechanical environment. In areas of high biomechanical stress, the synovium is flat and rests on a fibrous, mechanically strong subintima. This is particularly true of synovium underlying tendons that cross over a joint, such as the extensor tendon overlying the metacarpophalangeal joint. In synovial recesses, the intima is thrown into a three-dimensional villous architecture. This network is more richly vascular and has been shown to be a preferential site of solute and macromolecule exchanges.⁷ Hyaluronan synthesis is also greater in synovium from synovial villi.⁸

Intimal cells include synovial fibroblasts (or type B cells, approximately two-thirds of cells) and bone marrow-derived mononuclear phagocytes (or type A cells, approximately one-third of cells).⁹ Light microscopy techniques are not a useful guide for assessing lineage, as subintimal macrophages are often elongated and intimal fibroblasts may take a rounded

appearance.⁹ These cells can be separated on the basis of cytochemical staining for uridine diphosphoglucose dehydrogenase, a marker for hyaluronan synthesis, and non-specific esterase, a macrophage marker.¹⁰ These cells are also unique as they are found in close association with specialized components of the intimal matrix, such as fibronectin, laminin, type IV collagen, type V collagen, entactin, and sulfated glycosaminoglycans, which may serve to anchor the intimal cell layer to the underlying connective tissue.¹¹ The ability of the intima to maintain itself as a layer appears closely related to cell–matrix–cell interactions, through expression of adhesion molecules such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, fibronectin, laminin, and others, and their integrin ligands are likely candidates.

With joint overuse and inflammation, the relative predominance of these cell types is altered, with sub-intimal macrophages forming 50–70% of cells in the intima. Integrin expression is also increased and the morphology of the cells is changed from small cells arranged parallel to the joint surface to a more superficial location arranged perpendicular to the joint cavity. These cells are also the dominant source of intimal VCAM-1.⁹ Expression of cell adhesion molecules by synovial intimal cells serves to direct leukocyte trafficking in disease. Most multinucleate cells within inflamed synovial intima carry macrophage lineage markers.⁹ There is also evidence that synovial intimal cells could direct leukocyte trafficking in health and disease, through adhesion molecule expression.⁹ Intimal fibroblasts proliferate in disease consistent with the synovial proliferation observed with chronic joint use and inflammation.¹²

Joint circulation

The synovial membrane functions to maintain articular homeostasis by providing a pathway for the exchange of nutrients and metabolic byproducts between blood and synovial tissues, including articular cartilage.^{13,14} Optimal oxygen delivery to articular tissues serves to maintain normal synovial fluid composition,^{15,16} chondrocyte metabolism, and normal matrix composition and turnover.^{17–19} The efficiency of exchange between the synovial membrane capillaries and joint cavity is dependent on capillary density, capillary depth, and blood flow.^{15,20}

Several tissues in the joint are provided with a rich vascular supply that responds to exercise and pathological states. These include the joint capsule, synovial

membrane, intra- and periarticular ligaments, and subchondral bone. Articular cartilage is avascular. The articular blood supply of most diarthrodial joints is formed by small branches of the epiphyseal arteries running at the junction of the periosteum and the synovial membrane, forming an arterial circle. Larger branches penetrate the bone, whereas smaller branches remain at the periphery of the articular cartilage, forming the perichondral circulation. Subchondral bone blood supply is provided by the epiphyseal arteries, which travel in the epiphysis parallel to the articular cartilage, sending perpendicular branches which end in capillary loops at the deep surface of the calcified cartilage. Before physeal closure, this epiphyseal circulation is distinct from the metaphyseal circulation. The synovial membrane vascular supply is composed of capillaries which are very sparse in areas of high mechanical stress. The angle of reflection of the synovial membrane is composed of a rich vascular plexus and synovial villi are incompletely penetrated by a central arteriole.

The richest capillary density in the synovial membrane is within 2.5 mm of the joint surface. Capillary density is greatest in areolar or adipose synovium and lowest in fibrous synovium. Similarly, blood flow to synovium is greater than in the fibrous joint capsule and joint motion greatly affects intra-articular pressure and synovial blood flow (see Intra-articular pressure below).²¹ In addition, villous synovial membrane is more vascular than fibrous and preferential exchanges of small molecules such as albumin, as well as hyaluronan production, occur in synovial villi.⁷ Health of the synovial villi is important to maintain viscous synovial fluid rich in hyaluronan.²²

Factors that can acutely affect synovial blood flow include intra-articular pressure (IAP), local temperature, joint motion, vasomotor tone and reflexes, and local release of vasoactive mediators. Exercise is the greatest activator of joint circulation. Cardiac output is concomitantly increased, as is blood flow.^{23,24} Large arteries supplying the joint have intravascular pressure approaching systemic arterial pressure; however, blood flow and pressure are controlled at the arteriole and capillary level of the synovial membrane. Capillary pressure in the synovial membrane is low and local joint blood perfusion is strongly influenced by IAP. If effusion resulting in increased IAP is present, significant tamponade of the synovial blood flow occurs.²¹ Intra-articular pressures of >30 mmHg have been measured in fetlock joints of sound horses with effusion.²⁵ A significant decrease in blood flow to the synovial membrane was measured after an increased

IAP of 30 mmHg.²¹ Intra-articular acidosis develops at IAP of <45 mmHg.²⁶

Even in normal joint motion, as during exercise, regional blood flow will be arrested in accordance with high pressure profiles that occur within the compressed and highly tensed joint compartments.²⁷ As the joint moves from full extension to maximal flexion, a pumping action occurs, creating a pulsatile increase in blood flow.

Chronic joint disease, as seen in many active sport horses, can significantly decrease blood flow to the synovial membrane as the increased joint capsule fibrosis results in a concomitant decrease in capillary density and joint capsule compliance. Loss of joint capsule compliance increases IAP associated with joint effusion and motion.

Potential consequences of decreased articular blood flow include chronic ischemia and resultant synovial fibrosis and hypertrophy, generation of lactate and synovial fluid acidosis, and production of inflammatory mediators. Chronically hypoxic joints are more affected by exercise than normal joints, producing further hypoxia. A decreased blood supply could decrease drug delivery to the joint and decrease clearance of metabolites from the joint.

Morphometric and morphologic analyses of synovial vessels provide an insight into the contribution of synovial blood flow in disease.²⁸ Equine vessel anatomy contains a central arteriole and a helical venule descent from the tip to the base of the villus. The anatomical result is an arrangement that produces a countercurrent exchange system. These systems create ischemia at the tip of the susceptible villus²⁹ (Fig. 2.4.1). In normal synovial vessels, capillary flattening occurs with IAP greater than 25 mmHg.²⁸ In joint effusion and at phases of a normal stride, these IAP values are exceeded, resulting in decreased blood flow, decreased clearance and decreased lymph flow. These alterations are part of standard joint physiology in the horse in athletic training. In horses with chronic arthritis, decreased capillary density and increased intercapillary distance suggest altered trans-synovial fluid exchanges, as well as increased relative ischemia.

Data on joint physiology would suggest that in normal equine joints, an 'ebb and flow' of blood perfusion of synovial capillaries occurs from the back to the front of joints during exercise (Fig. 2.4.2). In joints with increased IAP (effusion) or decreased compliance (thickened stiff joint capsule), the reduction in blood flow will be even greater. The observation that synovial villi become blunted, shortened, and clubbed with chronic joint overuse further supports the scientific

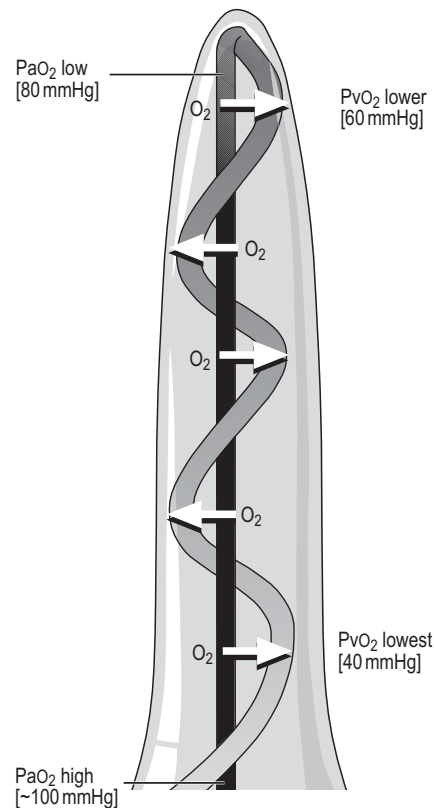


Fig. 2.4.1

Longitudinal section of an equine synovial villus. Note the central arteriole and helical venules that are anatomically appropriate for a countercurrent exchange mechanism and oxygen gradient from the arteriole to venules. The villus tip is susceptible to ischemia.

evidence that the tips of the villi are most susceptible to hypoxia in the form of the typical ischemia reperfusion injury.³⁰ The fibrotic thickening of the villi may be stimulated by oxygen gradients created during tissue hypoxia.

Intra-articular pressure

IAP is normally below atmospheric pressure in most joints at the 'angle of ease' and pressures between -2 and -12 mmHg have been reported. The 'angle of ease' is the most comfortable joint position and is typically a neutral position with the lowest IAP. Recordings of IAP in equine joints yielded similar subatmospheric values for the mid-carpal and metacarpophalangeal joints.^{25,28,31} Horses with healthy joints in active training will have 'tight' joints, i.e. no palpable effusion and a negative IAP. Maintenance of this negative pressure is thought to occur by joint

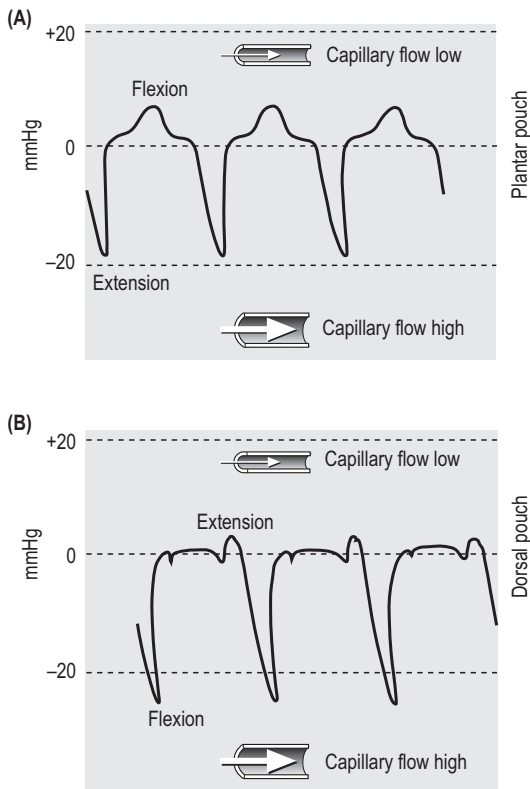


Fig. 2.4.2

Intra-articular pressure profiles of a normal exercising fetlock joint demonstrating the compartmentation and back-and-forth pulsatile blood flow in the capillaries of the synovial membrane. Capillary blood flow is higher during extension of the plantar pouch (A) and flexion of the dorsal pouch (lowest intra-articular pressure) (B), and lower during flexion of the plantar pouch (A) and extension of the dorsal pouch (highest intra-articular pressure) (B).

motion, which enhances lymph flow from the interstitium, and by joint flexion which promotes fluid absorption by raising IAP.

The normal fluid balance is therefore maintained through two pumps in series, one that enhances fluid exchange to the interstitium and one that enhances lymph flow. Examination of pressure–volume curves in normal joints indicates that this relationship is sigmoid, with low articular compliance at normal sub-atmospheric pressures, an increased compliance at supra-atmospheric pressures up to 30 mmHg, and another increase in compliance at high IAP.³¹ This relationship can be explained as a resistance to joint distension at IAP in the normal range, followed by accommodation of effusion at IAP up to 30 mmHg.

This may prevent collapse of synovium capillaries and preserve joint blood flow. At IAP >30 mmHg, the decreased compliance may counteract further effusion and articular fluid accumulation. Rupture of the mid-carpal joint capsule has been noted at IAP of >80 mmHg in horses and this rupture was located in the palmar lateral pouch of the mid-carpal joint, as has been observed clinically (Bertone, unpublished data, 1996).

The determinants of IAP include joint capsule compliance, joint angle, previous distension history, compliance of the joint capsule, muscle tension, and joint load.^{32,33} In addition, determination of pressure–volume relationships is also dependent on the type of infusate used for measurement.^{31,34} Chronically inflamed joints have thickening and fibrosis of the joint capsule, resulting in decreased compliance. Joint flexion increases IAP. Slow distension–compression cycles result in progressive stress–relaxation of the joint capsule and a gradual increase in articular compliance. However, if rapid successive infusion–withdrawal cycles are performed, a progressive decrease in articular compliance can be measured. If the joint is distended with synovial fluid, an increased compliance is observed compared to infusion with saline, probably because of the lack of a fluid interface which increases surface tension and promotes joint collapse, confirming the importance of hyaluronan in joint lubrication during training and exercise.

The effect of joint history dependence on IAP and compliance explains the lack of correlation between the volume of effusion and pressure generated from the effusion. Long-term slowly accumulating joint effusion will have relatively low IAP compared to fast-developing effusions. In addition, pain due to effusion is caused by periarticular tension receptors; stress–relaxation may explain why rapidly forming effusions are more painful than slower forming ones.

Compartmentation of the joint is the functional separation of joint compartments at physiologic pressure and has been demonstrated in the rabbit stifle and the equine metacarpophalangeal joint.²⁷ During joint movement, synovial fluid flows from compartments of highest pressure to compartments of lowest pressure, producing an ebb and flow of synovial fluid over the articular cartilage. This process provides nutrients to the avascular cartilage, lubricates cartilage and keeps joint pressures from escalating excessively during joint movement. Movement of fluid through the synovium into the interstitium and lymphatics (termed conductance) is increased with exercise. Both a direct effect of increased IAP and a pumping action on the interstitium and lymphatics

drive fluid resorption. Exercise increases this hydraulic conductance of fluid and improves clearance and turnover of joint fluid. This effect has been noted to continue for several hours after exercise. Normal exercise will not produce significant fluxes in IAP. These findings may be explained by the lower compliance of chronic diseased joints. In chronic joint effusion, pressures generated at rest but more remarkably at exercise are higher than capillary pressures, emphasizing the critical role of effusions on the generation of IAP.^{35,36}

Synovial fluid dynamics

Understanding the pathophysiologic mechanisms behind synovial fluid turnover becomes important when one considers the common concerns in actively

training sport horses, including joint effusion, the potential clinical use of synovial fluid markers as indicators of exercise or disease state and progression,^{37,38} and the use of systemic or intra-articular medications in the treatment of joint disease (Fig. 2.4.3). The histology, ultrastructure, and embryology of the synovium support the theory that the joint cavity is a third compartment of the interstitial space.³⁹ Interstitial and joint fluid is an ultrafiltrate of plasma to which hyaluronan is added. Fluid flow from the vascular space into the interstitium and joint space (third compartment space) and out into venules and lymphatics is tightly governed by Starling forces. Starling forces are a balance of arterial and venous pressures and colloid osmotic forces across the joint. The resultant fluid flow through the joint is modified by the permeability of the synovial membrane (osmotic reflection

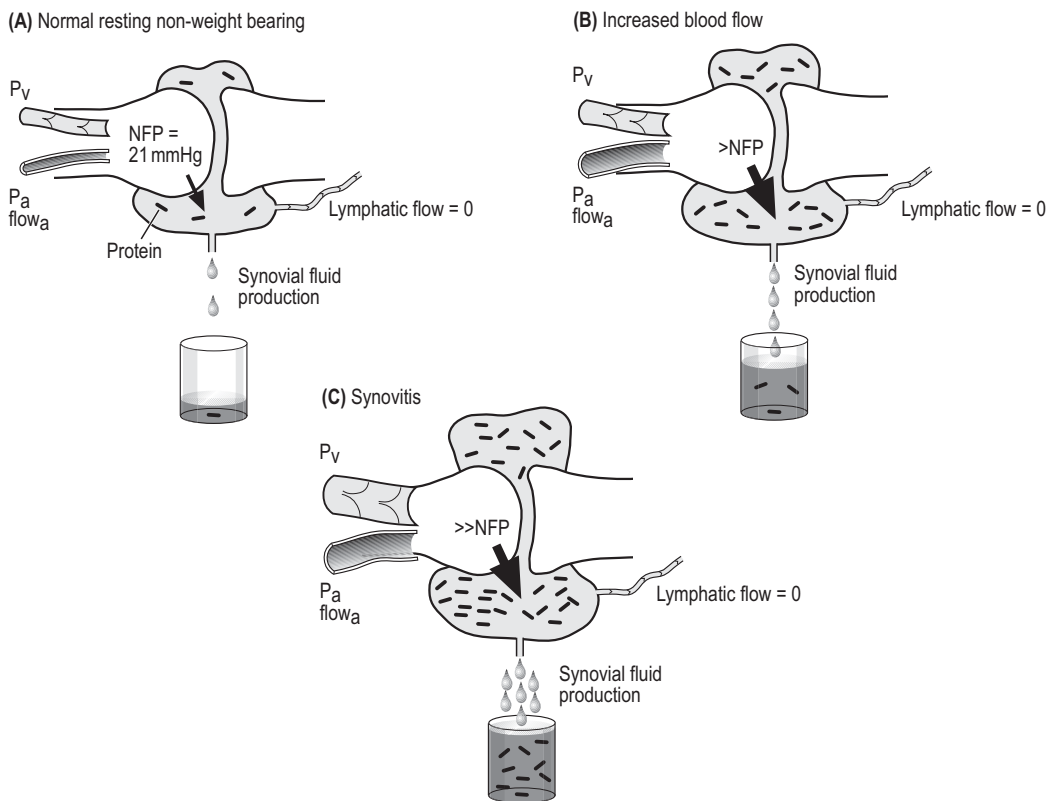


Fig. 2.4.3

Illustration of vascular and fluid forces in joints. (A) Normal joint at rest. (B) Normal joint during exercise with increased arterial blood flow. Arterial pressure and flow and trans-synovial flow are increased, but permeability is unchanged. (C) Abnormal joint with increased arterial blood flow such as occurs with synovitis. Arterial pressure (P_a) and flow (flow_a) and trans-synovial flow are increased. Permeability, and therefore synovial fluid protein concentration, and interstitial edema are also increased. NFP = net filtration pressure, P_v = venous pressure.

coefficient) and the vessel surface area available for fluid transport (filtration coefficient). Even in normal joints these forces are influenced by gravity (joint dependency), motion (exercise), and structure (joint compliance).^{1,6,20,27,38,40,41} Horses are also somewhat unique in necessitating joint motion to maintain isogravimetric states of the joints (no fluid gain or loss). This is most notable in peripheral joints.²⁰ In normal stationary or standing equine limbs, lymphatic drainage from joints approximates zero until joint pressure exceeds 11 mmHg for the fetlock joint (transitional microvascular pressure). In standing animals without counterforces, such as motion or external bandages to increase lymph flow forces, gravitational pressures both increase arterial pressure to the joint and increase the venous and lymphatic pressure necessary for fluid to exit the joint. The result is the tissue edema and joint effusion known as 'stocking up', a physiologic imbalance of joint fluid flow leading to a positive isogravimetric state of the joint (gain of weight in the form of interstitial fluid).

Articular albumin and hyaluronan (synovial coloids) are molecules that play a role in oncotic pressures and drive joint fluid dynamics.⁴² The half-life of the hyaluronan molecule in joints is relatively short (12–20 hours), suggesting that alteration in composition and molecular structure can quickly impact joint rheology. However, synovial fluid dynamics are dictated more by capillary permeability, IAP, and protein concentration than hyaluronan concentration.⁴³

Hyaluronan and joint lubrication

Hyaluronan is a non-sulfated glycosaminoglycan consisting of alternating units of D-glucuronic acid and N-acetyl-D-glucosamine. It exhibits polydispersity, but the average molecular weight is in the order of several million. In dilute solutions, each molecule behaves as a large coil but as the concentration of hyaluronan increases, entanglement of the coils occurs, eventually forming a uniform meshwork. Viscosity is non-linear and increases exponentially as the hyaluronan concentration increases, probably as a result of this three-dimensional framework.

The effect of hyaluronate on synovial fluid viscosity is proportional to chain length, protein concentration, pH, ionic composition, and temperature. Viscosity decreases rapidly with increased shear rate, such as during sustained high motion in competitive events. Viscosity is variable between joints, being high in small joints. In a given joint, viscosity varies inversely

with volume, and joint effusion usually dilutes hyaluronan and viscosity. The hyaluronan molecule has been assigned a role in providing joint boundary lubrication while its viscoelastic and shear dependence properties have only been slowly accepted functions. It is apparent, however, that the nature and configuration of the molecule, and the existence of receptors to hyaluronan, provide some other insights as to the role of hyaluronan *in vivo*.

Hyaluronan solutions provide a barrier against water flow and may act as a barrier against rapid tissue weight changes. The meshwork may also act as a sieve to regulate transport of macromolecules and exclude macromolecules from space in the system.⁴⁴ In articular cartilage the hyaluronan-binding proteins, also referred to as hyaladherins, are aggrecan and link protein, which in combination with hyaluronan form the large proteoglycan aggregates of articular cartilage which provide the compressive stiffness necessary to accept loads such as occur in athletic events. Synovial fluid hyaluronan is also critically important to the mechanical properties of the joint cavity, enhancing the compliance of the joint capsule.³¹

Hyaluronan has also been shown to decrease joint pain with molecular weights of greater than 40 kDa. Hyaluronan of 860 kDa and 2300 kDa produced high-level and long-acting analgesia for 72 hours after injection. This effect was not related to binding to hyaluronan or bradykinin receptors.⁴⁵

Joint pain

Joint pain is an important component of training any equine athlete, as lameness is the greatest cause of morbidity in horses.⁴⁶ The physiology of joint pain primarily involves pain fibers found in the synovial membrane or subchondral bone. Joint pain can usually be attributed to joint inflammation, restrictive fibrosis, and/or subchondral bone pain. Excessive joint strain during exercise can be in the form of a singular mishap or repetitive overstrain leading to joint inflammation. The joint is richly innervated with large myelinated afferent and efferent nerve endings and small unmyelinated C fibers. Sensory and motor innervation provides feedback that helps maintain joint stability, such that in the absence of these protective reflexes, severe arthropathy may develop if the joint is made unstable.⁴⁷ Activation of the peripheral nervous system can initiate the major features of acute inflammation, which include vasodilation, effusion, and a lower

threshold for pain.^{48,49} The pain of arthritis is relayed by both C fibers and A δ (delta) fibers. These fibers are activated by amines (serotonin, etc.) and neuropeptides (CGRP and substance P) that act synergistically to exert proinflammatory effects on the synovium. The presence of these neuropeptides has been documented in equine articular tissues.

Although acute inflammation is a necessary and appropriate response to initiate repair following tissue injury, inadequate regulation of this response may lead to excessive tissue damage or chronic inflammation. A role of substance P in joint pain is supported by the clinical effectiveness of the substance P-depleting substance, capsaicin. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the pungent ingredient in hot paprika or chili peppers. It initially activates C fibers, resulting in substance P release and pain, but subsequently desensitizes or degenerates C fibers, suggesting a mechanism for pain alleviation with chronic use in articular inflammation.⁵⁰

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of articular inflammation and in the control of joint pain in athletic horses in training. The role of prostaglandins in pain is indirect, as they act to sensitize C fibers to subsequent stimulation. NSAIDs inhibit prostaglandin synthase enzymes (also known as cyclo-oxygenase) and diminish the formation of PGE₁, PGE₂, PGF, and PGI from arachidonic acid. Corticosteroids inhibit phospholipase A₂, thus preventing the formation of arachidonate, a substrate for the cyclo-oxygenase and lipoxygenase pathways. At high doses, corticosteroids also inhibit interleukin (IL)-1 and tumor necrosis factor (TNF), which can also sensitize pain nociceptors. These mechanisms may explain the analgesic effects of NSAIDs and steroids.

The role of neuropeptides in joint disease is currently under investigation and there appear to be differences in the contribution of neuropeptides to disease process in acute versus chronic inflammatory arthritis. In acute arthritis, loss of sensory nerves may contribute to inflammation, as demonstrated by increased edema formation in denervated limbs.⁵⁰ Similar results have been reported in an IL-1 induced model of acute inflammation in the horse, where increased edema and decreased permeability to macromolecules were observed in denervated limbs.⁵¹ The role of innervation in chronic arthritis is complex. Staining for CGRP and substance P was increased in the sciatic nerve, dorsal root ganglia, and periarticular tissues, but synovium staining was decreased. It appears that the role of neuropeptides in acute or chronic

inflammation may vary as the distribution of sensory nerves is altered with the inflammatory response.

The therapeutic implications of the participation of neuroendocrine mechanisms in arthritis are many. Intramuscularly administered gold or topically applied capsaicin are agents that selectively destroy C fibers, thus lowering substance P levels, and have been found clinically useful. Capsaicin initially causes release of substance P from nerve endings, explaining the burning sensation felt upon initial application. NSAIDs (PGH₂ or cyclo-oxygenase inhibitors) decrease prostanoic acid production, and intra-articular corticosteroids, which inhibit the arachidonic acid cascade, are effective in the treatment of inflammation and pain in arthritis.⁵² In addition, stimulation of primary afferent nociceptive fibers causes release of glutamate and substance P from central spinal pathways. This nociceptive input can be inhibited by stimulation of proprioceptive and tactile type I and II fibers. Stimulation of these fibers can be accomplished by high-frequency, low-intensity transcutaneous neural stimulation, frequently used in physiotherapy.

Cartilage adaptation to training and exercise

Adaptation of cartilage to exercise is well established in horses and results in cartilage able to handle greater biomechanical stress, particularly in anatomical sites receiving high loads.⁵³⁻⁶⁹ Joint stress and osteoarthritis are correlated in people and are empirically correlated in the equine athlete as well.⁷⁰ Overuse results in wear and tear when the stress of exercise exceeds the capability of the cartilage to adapt and structural damage occurs. Virtually all elite equine athletes with an extended career will have some degree of osteoarthritis. Exercise both increases and accelerates the development of biochemical articular cartilage heterogeneity.^{55,58,65} These changes reflect the biodistribution of loading⁵³ and loading-induced changes in synovial fluid.⁶² Use of intra-articular steroid medication in exercising horses has been shown to reduce the biomechanical supportive properties of the equine articular cartilage.⁶⁷

Some of the differences in biomechanical and biochemical properties of articular cartilage result from species disparities, the characteristics of a particular joint, or as a function of location within a joint. Differences in similar anatomic site locations in joints as a function of the level of exercise (i.e. non-strenuous as

compared to strenuous) demonstrate that the history of loading undergone by the joint alters these biomechanical material properties. Indentation studies on equine articular cartilage from exercised and non-exercised horses demonstrated clear differences in bi-material properties of the cartilage which were site-specific. Sites of higher loading had greater changes, indicating an exercise adaptation. This was most dramatic for cartilage permeability (fluid conductance) in which exercise promotes water (fluid) flow out of the cartilage on loading. Fluid extrusion from articular cartilage on high-impact loading is a known mechanism for cartilage lubrication.⁴¹ These biomechanical adaptations of cartilage correspond in a site-specific manner to alterations in cartilage metabolism with exercise. Chondrocytes increase their production and quality of proteoglycan to increase the compressive stiffness of cartilage. It takes longer than 3 weeks of training for the increase in proteoglycan synthesis to result in a measurable increase in total proteoglycan content.⁵⁴

There is no consensus on the influence of exercise (beneficial⁷¹ or neutral⁷²) on the healing of injured articular cartilage, although assimilation of the studies would suggest that exercise during healing is beneficial as long as the impact trauma is below the level of repair tissue destruction.

Bone adaptation to training and exercise

In young horses put in training, bone is exposed to new stresses. During training bone rapidly remodels to decrease bone porosity and increase bone trabecular width and mineralizing surface, thereby enhancing the bone's ability to withstand stress.⁷³ Younger horse bone (2-year-olds) is less stiff and therefore greater strains (bone movement) have been measured during high-speed exercise as compared to older horses. These high strains seen in these young horses may lead to high-strain, low-cycle fatigue of the bone and subsequent bone pain (dorsal metacarpal cortex, sesamoid bones, caudal metacarpal condyle).⁷⁴ Computed tomography and histology have confirmed the presence of microcracks at these high-strain bone sites. Bone strain is even greater in the lead limb (the left limb in North American racing), correlating with the most common location of ultimate fracture (dorsal cortical fracture and condylar fracture).⁷⁵

Immature cortical bone of horses is normally resorbing primary osteons during training and has greater resorption cavities and incompletely filled secondary osteons than that of older horses. This bone structure is more susceptible to fatigue micro-damage resulting from training because of higher bone porosity, fewer completed secondary osteons and a lower proportion of circumferentially oriented collagen fibers.⁷⁶ Indeed, in racing Quarter Horses put in training, the bone density significantly decreased early in training and then increased later in training. Race horses experienced fewer bone-related injuries when they had greater cortical mass in areas of known high bone stress at the commencement of training.⁷⁷

The metacarpus changes shape during maturation⁷⁸ and training^{79,80} to lower strains during high-speed exercise. As an example, the dorsal cortex thickens during training, by production of periosteal new bone. This natural response to these demands on the bone enables bone to handle stress without developing microfractures or complete bone failure (fractures). Experimental exercise conditions confirm marked modeling (not remodeling) of the bone, particularly subperiosteal bone formation at the midshaft of the third metacarpal bone. Horses that complete a full training program have greater bone mineral content despite a lighter body weight, further demonstrating the principle of Wolfe's Law, summarized as the principle that bone is deposited in areas of increased bone stress demands.⁸¹ Bone mineral density increases with age and exercise is critical for normal bone development.⁸²

In summary, in horses in training, high bone strain can induce cyclic fatigue of bone, resulting in micro-damage and ultimate bone failure. The less bone present at the start of training (immaturity or lack of musculoskeletal conditioning), the greater this risk. Bone responds by modeling but microfracture damage may develop and cause pain. Many (>80%) 2-year-old racing Thoroughbreds⁸³ and Quarter Horses⁸⁴ demonstrate bone pain and it is estimated that ~12% go on to develop stress fracture, usually within 6 months to 1 year of showing pain.⁷⁸ Horses trained on harder surfaces (dirt as compared to wood fiber)⁸⁵ and faster horses⁸³ are at greater risk of developing bone pain and microfracture. It is proposed that the greater incidence of bone fatigue failure in Thoroughbreds, as compared to Standardbreds, is due to gait differences and resultant bone stresses during training and racing, not to inherent differences in the mechanical properties of the bone.⁸⁶

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Biomechanics of locomotion in the athletic horse

Eric Barrey

Introduction

The horse is a superathlete which often suffers from injuries of its locomotor apparatus because of human management errors (nutrition, training, shoeing, breeding), bad environmental conditions (tracks, weather), and/or an unfavorable constitution (limb conformation, genetics). In stables specializing in gallop racing, about 53–68% of the wastage in race horses is due to lamenesses.^{1,2} This economical disaster justifies the great effort now being put into equine locomotion research, including clinical applications and techniques for preventing lameness. Currently, economic constraints also favor the development of early performance evaluation in order to improve the training and selection of young horses.

This chapter presents a review of equine locomotion and the applications of gait analysis. Current knowledge concerning the equine locomotion variables in various sporting disciplines and the influence of training are discussed. Finally, a survey of the practical applications of equine gait analysis will be presented.

Locomotion analysis

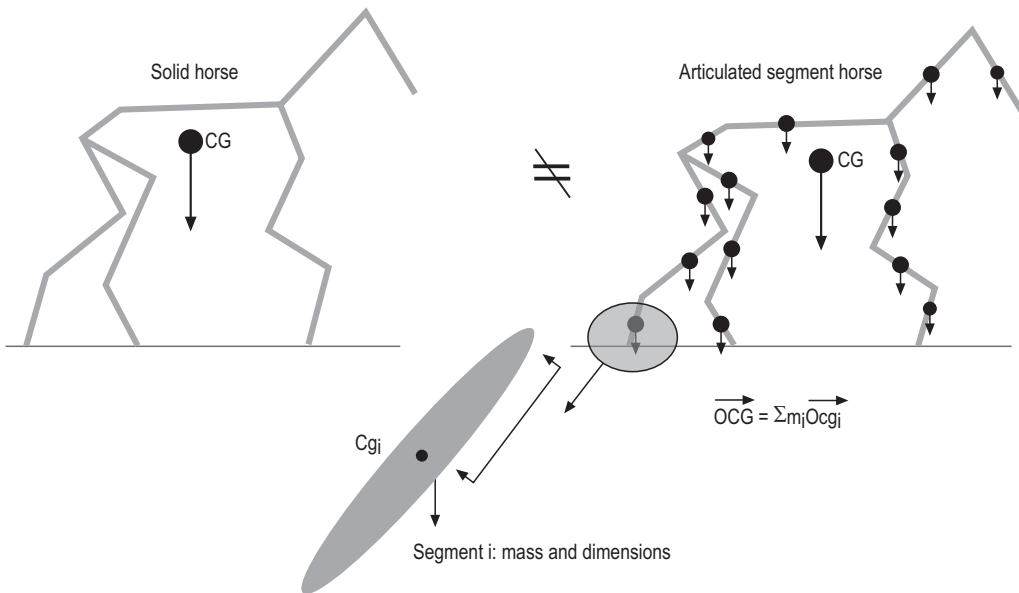
The body of the horse is composed of a set of rigid segments articulated one to another. Consequently, the body of the horse follows exactly the same mechanical laws as a series of inanimate objects (Fig. 2.5.1). However, these laws need to be applied carefully because the mechanical equations which determine the motions of a set of articulated body segments are much more complicated than those that determine the motion of a single inanimate object like a bullet. There are two complementary methods for studying

the body in motion: kinetics and kinematics (Box 2.5.1).

- *Kinetics or dynamics* studies the cause of the motion, which can be explained by the force applied to the body, its mass distribution, and its dimensions. Kinetics is concerned with forces, energy, and work, which are also related to kinematic variables such as acceleration and velocity. Acceleration can be directly measured by specific sensors. However, acceleration is defined as an instantaneous change of velocity (i.e. the derivative of the velocity against time). Consequently, it can be deduced from velocity data obtained in kinematics by displacement measurements.
- *Kinematics* studies the changes in the position of the body segments in space during a specified time. The motions are described quantitatively by linear and angular variables which relate time, displacement, velocity, and acceleration. No reference is made in kinematics to the cause of motion. The kinematic approach is more commonly employed, probably because it is easier to measure and visualize some displacements or velocities than to measure and imagine some forces, moments, or accelerations applied to the body.

Kinematic analysis

The use of chronophotography was first developed by Muybridge and Marey for animal locomotion analysis. Currently new technology, using high-speed cameras (16 mm, 500 images/s), is used, for example, to film the locomotion of Standardbred horses from a camera car under track conditions.³ Markers are used which are composed of small white spots or half spheres glued on to the skin over standard anatomical

**Fig. 2.5.1**

A horse mechanical model composed of articulated body segments. It is very different from a mechanical model with only one solid segment. For each segment (i) of the body, the center of gravity (cg) and the moment of inertia can be calculated. The location of the general center of gravity (CG) of the horse can be calculated by considering the mass and the coordinates of each center of gravity segment. (Reproduced from Barrey E. Methods, applications and limitations of gait analysis in horses. *The Veterinary Journal* 1999; 157:7–22.)

Box 2.5.1 Advantages and limits of the methods

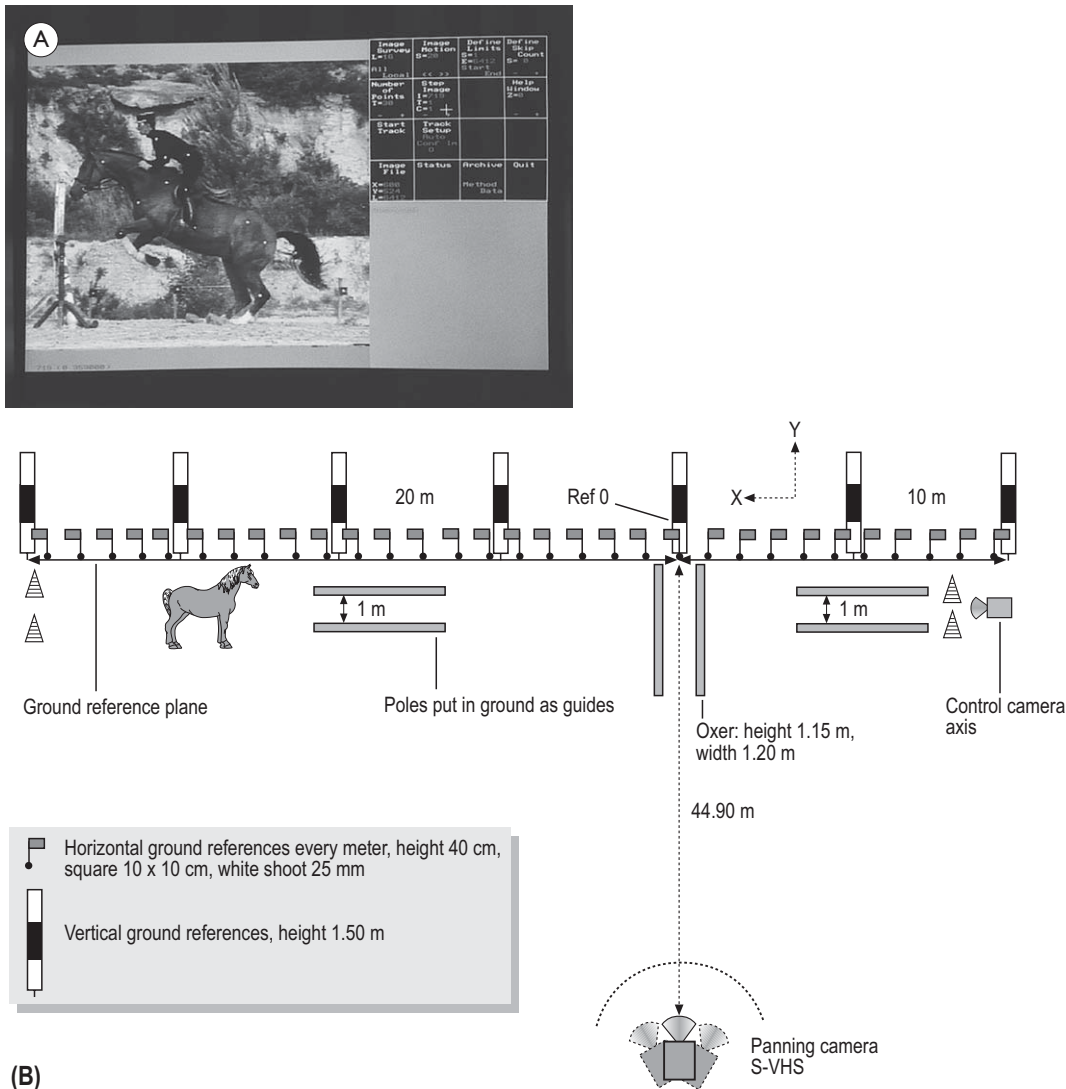
Kinetics	Kinematics
Explain cause of motion	Describe the motion
Forces, kinetic moments, accelerations, work, energy	Trajectories, angles, velocities, accelerations
Transducers → signals	Images → coordinates
Rapid analysis	Time-consuming
Synthetic information	Details of the movements
Physical sensitivity	Visual

locations (Fig. 2.5.2A).^{4,5} They are intended to indicate the approximate instantaneous center of rotation of the joint.^{6,7} However, the skin displacements over the skeleton during locomotion generate some artifacts, especially in the proximal joints.^{8,9}

The processing of the film for collecting the joint marker coordinates is undertaken using a computer. This is a relatively time-consuming task but many time and linear characteristics of the strides can be

obtained for describing individual gait variations. With the improvement of the video camera sensors (CCD) many professional high-speed video cameras (100–2000 images/s) and home video cameras (PAL or NTSC Standard: 25–30 images/s or 50–60 frames/s) can be employed for locomotion analysis. The video signal can be treated by a video interface in order to digitize the images, which are then analyzed by the appropriate software to collect semiautomatically or automatically the marker coordinates in space and time.¹⁰ A more sophisticated motion analysis system uses active markers which consist of photodiodes (modified Cartesian optoelectronic dynamic anthropometer; CODA-3). The advantage of this system is its good resolution (0.2–2.6 mm) in three dimensions, high recording frequency (300 Hz) and the automatic tracking possibilities of the active markers.¹¹ The main disadvantage is that the subject needs to be equipped with many photodiodes connected to wires.

Most equine locomotion studies show two-dimensional motion analysis but some systems with four or more video cameras make it possible to reconstruct the motion in three dimensions and to analyze the limb motions of both sides.^{12,13} One limit of these sophisticated gait analysis systems is the restricted

**Fig. 2.5.2**

Example of two-dimensional kinematic study of jumping horse. (A) One image extracted from the video film shows the horse and rider equipped with white anatomic markers. These markers are used to identify the location of each joint on each image. (B) Film recording procedure for a panning camera system to film and analyze the motion of a large field (30 m) as in jumping exercise. A set of ground reference planes was placed parallel and behind the horse trajectory.

field of view. This is only about 5 meters, which corresponds to several walking strides or one trotting stride. In order to analyze sporting exercise in a wider field (up to 30 m), a camera panning technique has been developed and used to study gait parameters in dressage and jumping horses.^{10,14,15} This technique can be used for the kinematic analysis of athletic locomotion under real exercise conditions (Fig. 2.5.2B).

After filming, the operator needs to track manually, semiautomatically, or automatically, the coordinates of the markers on each image of the film. In most of the systems, the tracking phase is a long task because there are many images to analyze, and as the markers are not always easy to detect automatically, manual supervision is required. Nowadays, the use of specific algorithms such as direct linear transform (DLT) is an efficient way to determine automatically the

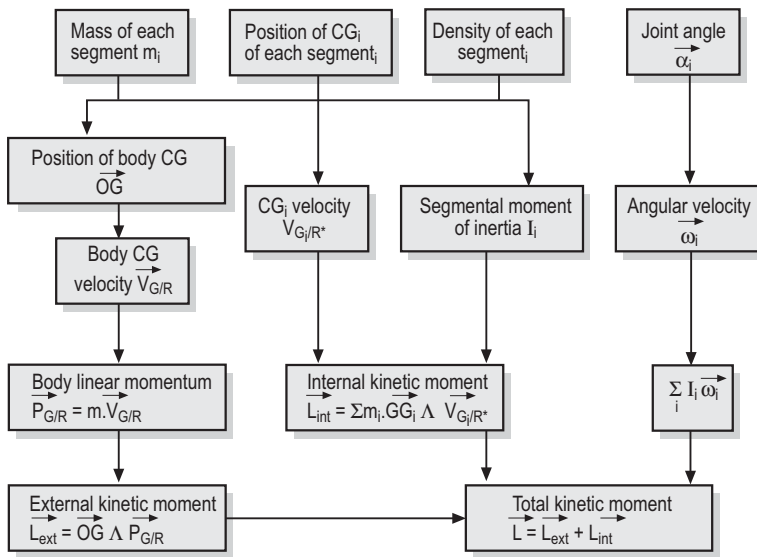


Fig. 2.5.2—continued

(C) Data analysis procedure to compute the center of gravity (CG) and total kinetic moment (L). After tracking the markers of each joint, the coordinates data were used to calculate the center of gravity (CG_i) of each segment, the velocity (V_g), the moment of inertia (I_i), the angles (a_i), the angular velocity (v_i), and the external and internal kinetic moment. (Figure 2.5.2B, C reproduced from Galloux & Barrey¹⁵ with permission.)

trajectory of the markers. This makes it possible to use these systems for practical applications such as lameness quantification or athletic gait evaluation.

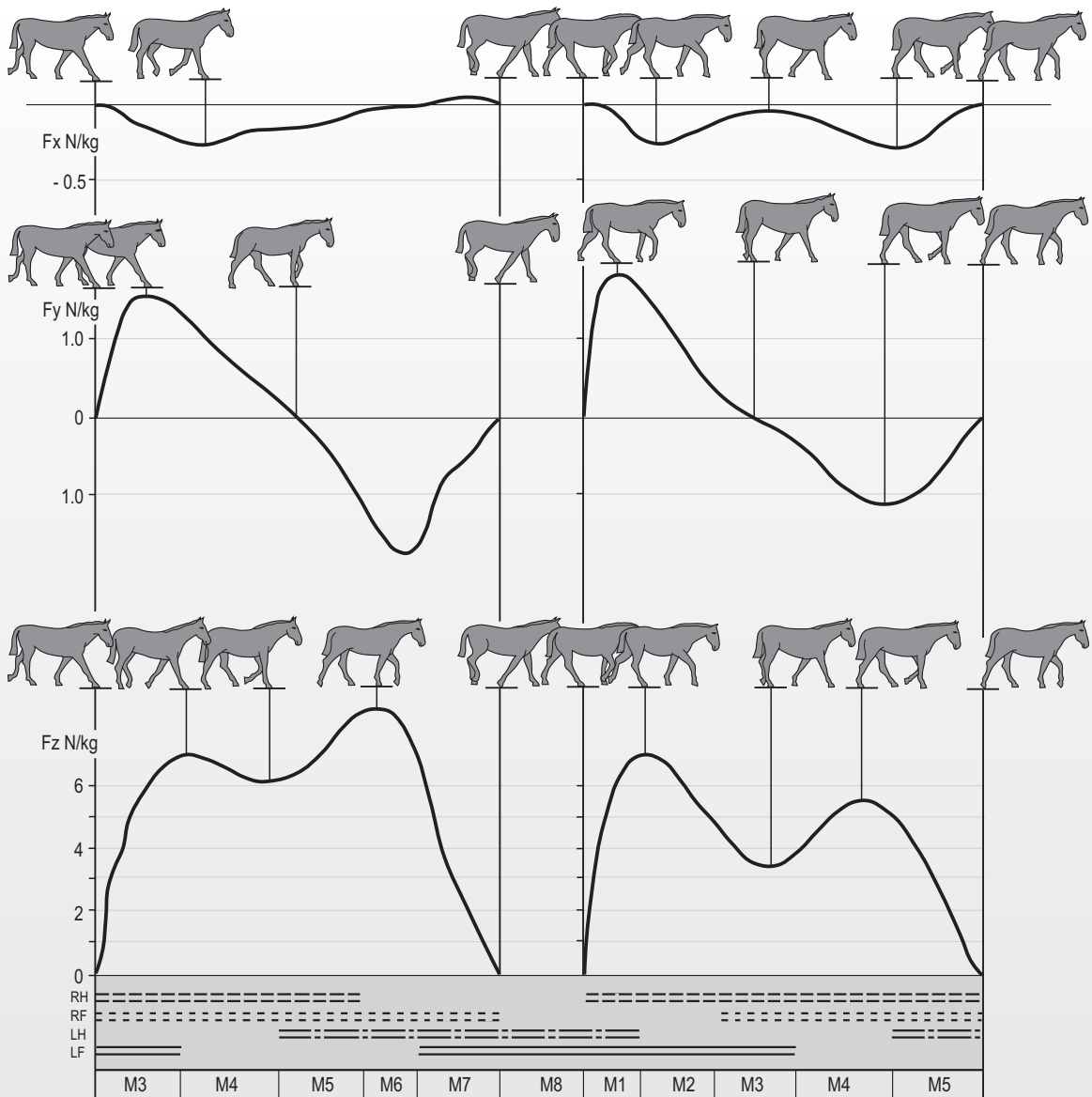
After collecting the coordinates of the markers, the linear and angular velocities can be obtained by computing the first derivative of the trajectories and angles with respect to time. If the filming image frequency is high, the second-order derivative of a trajectory or angular variation with respect to time, using appropriate smoothing and filtering techniques, provides linear and angular acceleration data. The advantage of kinematics methods is that you can obtain all the kinematic parameters (displacement, velocity, linear acceleration, angle of rotation, angular velocity, and angular acceleration) of the identified segments. If the center of gravity and the moment of inertia of each segment can be determined by measuring their mass distribution and dimensions, it is possible to calculate the kinetic parameters (forces and kinetic moment), which determine the motion of each segment, from the kinematic data. Finally, the kinetic energy can be estimated for each segment and for the whole body in motion. Several methods have been described to estimate the location of center of gravity and the moment of inertia of each segment (Fig. 2.5.2C).^{15–17}

Kinetic analysis

Another approach to the study of the biomechanics of locomotion is to measure either the external forces applied to the body or the accelerations of the center of gravity of the body segments. Marey (1873) was the first author to use a pressure sensor attached to the ground surface of a horse shoe and accelerometers attached to the limbs to measure the hoof–ground contact durations at the various gaits.¹⁸ All the sensors measured forces using pneumatic principles. The variations in pressure generated by the various transducers were recorded by tracing curves with a portable pneumotachograph.

Ground reaction forces

Modern sensor technology is much improved and is capable of making accurate measurements over a large range of conditions. However, the measurement principles have remained identical. The external forces are measured using electronic force sensors which record the ground reaction forces when the hooves are in contact with the ground. The sensors can be installed either on the ground in a force plate device

**Fig. 2.5.3**

Limb positioning at the time of characteristic ground reaction force amplitudes of the right fore- and hindlimbs of a clinically sound Dutch Warmblood horse at normal walk. The phases of the concurrently loaded limbs are presented in a bar diagram. (Reproduced from Merckens²³ with permission.)

or in a shoe attached to the hoof. The force plates can provide the force amplitude and orientation (vector coordinates in three dimensions; Fig. 2.5.3), the coordinates of the point of application of the force, and the moment value at this point.^{19–23} The accuracy of this type of device is usually good, but the sensitive surface is rather small (about 0.5 m²) and a visual control of the hoof trajectory is required.

In human biomechanics, the treadmill has been used to measure vertical ground reaction forces in standardized exercise conditions. In equine biomechanics, vertical ground reaction forces are measured for all four limbs simultaneously with the treadmill integrated force measuring system.²⁴ It is used for lameness evaluation in standardized conditions of speed, hardness of the ground, and environment.²⁵

**Fig. 2.5.4**

(A) Horse shoe for measuring vertical ground reaction forces. (B) The hoof was supported by four force transducers (strain gauges, Wheatson bridge) in order to measure hoof force distribution between heels, quarters, and toe.

In order to measure the ground reaction forces during various exercises, several authors have developed hoof force shoes including one or several force sensors (Fig. 2.5.4).^{18,26–30} Depending on their design, these devices can give between one and three components of the ground reaction forces and the point of application. They are generally less accurate than the force plate and their main disadvantage is the additional weight and thickness of the special shoe. Recently, another indirect ambulatory technique of ground reaction force evaluation was proposed using strain gauges glued on to the hoof wall. After the training of the appropriate artificial neural networks, the ground reaction forces can be estimated from the hoof wall deformations.³¹

Acceleration analysis

Acceleration analysis is a kinetic method that measures instantaneous change of velocity which is produced by applying a force on a solid during the same duration. Acceleration measurements are performed using small sensors (accelerometers) which should be firmly attached to the body segment under study. These sensors are made of a small suspended mass giving a signal which is proportional to the acceleration. A sudden change in velocity can give a high acceleration or deceleration (decrease of acceleration), even if the displacement is small. The acceleration vector is proportional to the resultant force applied to the body's center of gravity and its measurement provides a convenient way to study the kinetics of a body in motion.

In order to analyze horse locomotion, the accelerometer should be placed as near as possible to the body center of gravity. The caudal part of the sternum between the right and left pectoralis ascendens muscles at the level of the girth provides a good compromise between transducer stability and closeness to the horse's center of gravity (about 65 cm dorsocaudally at the gallop). The acceleration signal is transmitted to a PC or recorded with a small data logger placed in the saddle pad (Fig. 2.5.5A). The first application of this gait analysis system (Equimetrix TM) was used for harnessed trotters evaluation.³² The acceleration signal, such as dorsoventral acceleration of the trot (Fig. 2.5.5B), could be treated by signal analysis procedures in order to extract the dynamic and temporal stride variables. Calculating the double integral of the linear acceleration makes it possible to find kinematic variables (linear or angular displacement) such as the instantaneous displacement of the saddle in space.³³ Several examples of gait variables calculated from acceleration data will be presented in the following paragraphs.

Acceleration measurements could also be employed for analyzing the energy characteristics of shocks and vibrations which are transient in the hoof.^{34–36} An accelerometer could be fixed on the hoof wall in order to measure the maximal deceleration of the hoof impact on the ground and the vibration frequency (Fig. 2.5.6). The influence of horse shoes and ground surface characteristics could be studied using this method.

The main advantage of using an accelerometric transducer is the simplicity of the measuring technique. It can easily be used under field conditions. The main limitation is that the measurements are given

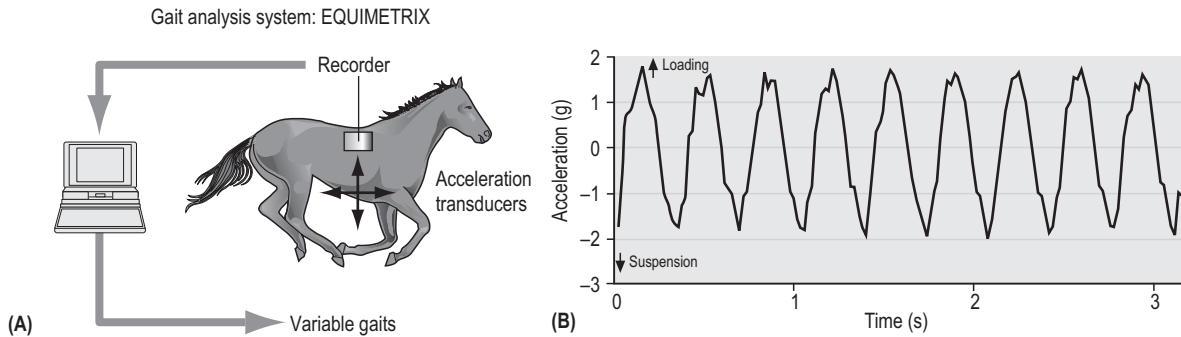


Fig. 2.5.5

Accelerometric Equimetrix gait analysis system. (A) Two- or three-dimensional accelerometers are fixed on the sternum by an elastic belt or the girth of the saddle. The accelerations are recorded continuously during the exercise. Then data analysis calculates gait characteristics such as stride frequency, vertical and longitudinal activity, regularity, and other gait variables specific to the exercise. (B) Example of the vertical acceleration recorded at the trot. The peaks correspond to the maximum loading of the diagonal. The valleys correspond to the suspension phases.

with respect to a set of body axes and consequently it is not easy to calculate the acceleration, velocity, or displacement values with respect to a set of ground axes.

Conditions of gait measurements: treadmill exercise versus ground exercise

Under laboratory conditions it is possible to study the locomotion of horses running on an experimental track or on a treadmill. The latter provides an excellent means of controlling the regularity of the gaits because the velocity and slope of the treadmill belt are entirely fixed by the operator. In order to analyze the gait of a horse without stress, some pre-experimental exercise sessions are required to accustom it to this unusual exercise condition.³⁷ The horse adapts rapidly at the trot, and stride measurements can be undertaken beginning at the third session. For the walk, many stride parameters are not stable, even after the ninth training session. Within a session, a minimum of 5 min of walking or trotting is required to reach a steady state of locomotion.

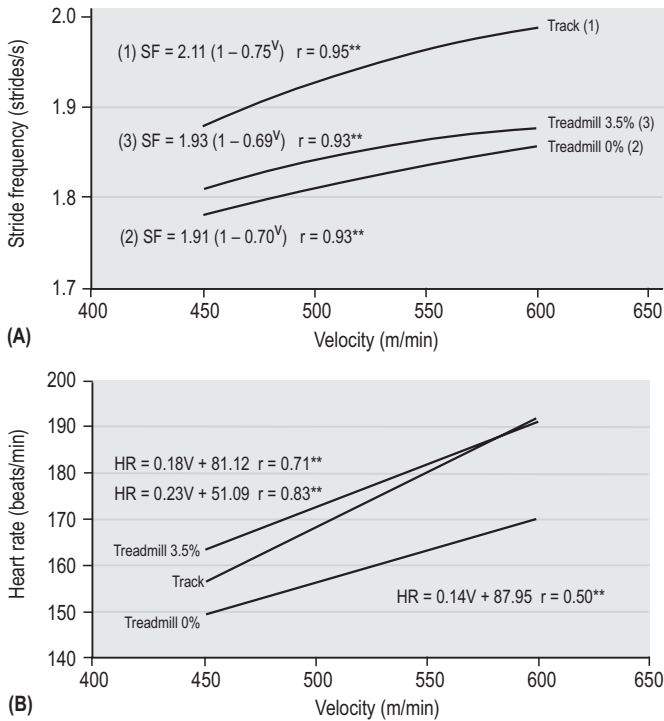
Many fundamental locomotion studies have been performed on commercially available high-speed treadmills, since the development of the first installation of this type of machine at the Swedish University of Agricultural Science in Uppsala.³⁸ At the beginning of human treadmill use, it was suggested that



Fig. 2.5.6

Accelerometer fixed on the hoof to study the shocks and vibrations of the hoof after impact on the ground. The influence of the ground surface and the shoe on the shock damping was studied using this transducer.

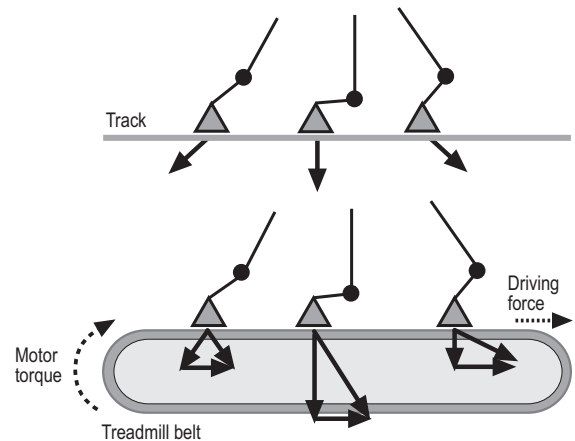
locomotion on a treadmill would be exactly the same as on the ground.³⁹ This hypothesis did not take into account the fact that the human body is not a rigid body system but an articulated set of segments. In horses, it was demonstrated experimentally that the stride parameters are modified in flat and inclined

**Fig. 2.5.7**

Comparison of the stride frequency (A) and heart rate (B) of horses galloping on track, flat treadmill, and 3.5% inclined treadmill. At the same velocity, the stride frequency is lower on the treadmill (i.e. stride length longer) than on the track. The heart rate response is the same on track and 3.5% inclined treadmill. (Reproduced from Barrey et al. *Equine Athlete* 1993; 6:14–19, with permission.)

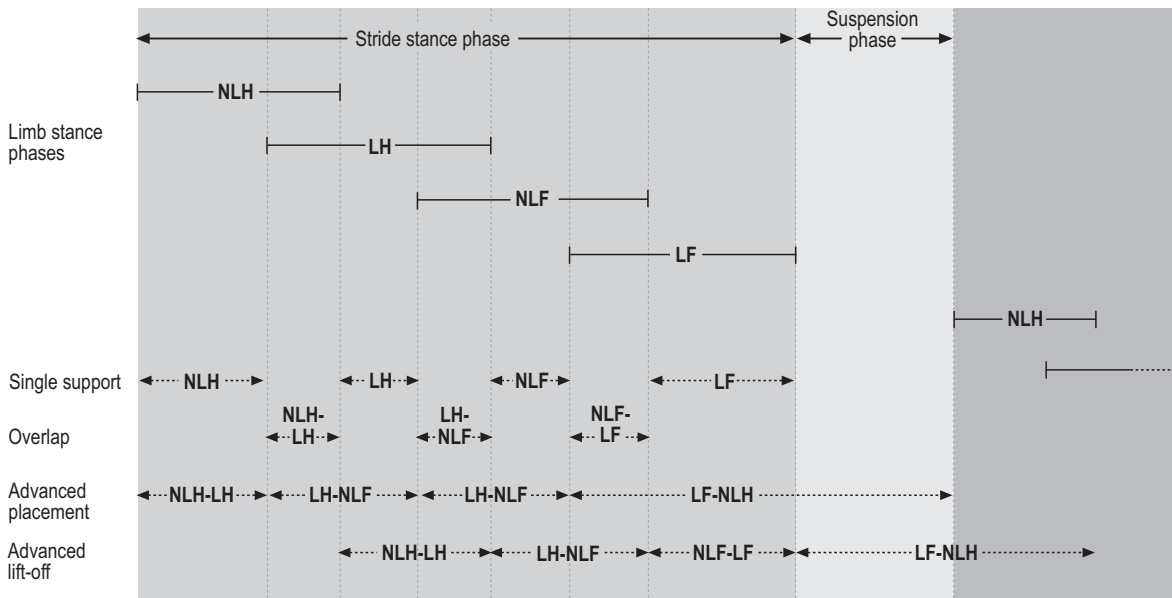
exercise at trot and canter.²³ At the same speed, the stride frequency was lower on the treadmill and the stride length was longer than in overground conditions (Fig. 2.5.7A). The exercise on a flat treadmill generated a lower cardiac and blood lactate response than exercise on the track at the same velocities.^{23,40} In Trotters tested under two training tracks and treadmill conditions, there were no significant differences in locomotor and physiological variables between tracks but the treadmill had the same influence as in saddle horses.⁴¹ In order to reproduce approximately the same energetic exercise on a treadmill, it was found that a treadmill incline of 3.5% gives the same heart rate response as in overground conditions (Fig. 2.5.7B).

The mechanical reasons for these differences are still not entirely understood but some explanations have been suggested by the experimental and theoretical results. The treadmill belt is driven by the motor and it helps the horse's limbs to move backwards (Fig. 2.5.8). The speed of the treadmill belt fluctuates in relationship to the hoof impact on the belt.³¹ The total kinetic energy of human runners filmed at the same speed on flat track and treadmill was calculated using the kinematic data (all the body segments taken into account). It was found that the total kinetic energy was reduced by a factor of 10 on the treadmill compared with on the track.⁴² This difference was

**Fig. 2.5.8**

Schematic diagram of hoof force differences in overground (top) and treadmill (bottom) locomotion. The additional force of the treadmill motor acts on the hoof, which could explain the decrease of work and increase of stride length observed in treadmill exercise. (Reproduced from Barrey et al. *Equine Athlete* 1993; 6:14–19, with permission.)

mainly explained by a reduction of the kinetic energy of each limb and arm segment, which moved with a lower amplitude around the total body center of gravity on the treadmill as compared with track conditions. However, these results cannot be extrapo-

**Fig. 2.5.9**

Example of temporal stride variables at the gallop. Bars indicate the stance phase of the limbs. NLH, non-lead hindlimb; LH, lead hindlimb; NLF, non-lead forelimb; LF, lead forelimb. (Reproduced from Clayton⁴⁹ with permission.)

lated to the horse because the measurements and calculations do not relate to quadrupedal locomotion.

At a slow trot, a 6% inclination of the treadmill tends to increase the stride duration and significantly increases the stance duration of the forelimbs and hindlimbs.⁴³ Kinematic analysis has confirmed that the hindlimbs generated higher propulsion work on the inclined, rather than flat, treadmill. The inclination of the treadmill did not change the stride length nor did it change the stance, swing, and stride duration in a cantering Thoroughbred.⁴⁴

Response to exercise: velocity-related changes in gaits and stride characteristics

Gait terminology and definitions of stride characteristics

Gait variety and complexity have always created difficulties because of the need for adequate terminology to describe the locomotor phenomenon. Some efforts have been made to define a standard terminology for describing equine locomotion.^{45–47} A *gait* can be defined as a complex and strictly coordinated rhythmic and automatic movement of the limbs and the

entire body of the animal which results in the production of progressive movements. A two-, three- or four-beat gait corresponds to the number of hoof impacts that can be heard during a stride of trot, canter, and gallop, respectively. The sounds are related to the foot-fall sequence of the gait but small asynchrony cannot be detected audibly. To describe the footfall sequence of each gait, it is useful to name the four limbs: right forelimb (RF), left forelimb (LF), right hindlimb (RH), and left hindlimb (LH). Many methods have been proposed to describe more precisely in time and space the limb movements: drawings, chronophotographies, stick bar (Fig. 2.5.9), phase, and pie diagrams.^{18,48–50}

The *stride* is defined as a full cycle of limb motion. Since the pattern is repeated, the beginning of the stride can be at any point in the pattern and the end of that stride at the same place in the beginning of the next pattern. A complete limb cycle includes a stance phase when the limb is in contact with the ground, a swing phase when the limb is not in contact with the ground, and a suspension phase when none of the hooves is in contact with the ground. The stride duration is composed of the stride stance phase (total duration of ground contact) plus the suspension phase. It is also equal to the sum of the stance and swing phase duration of one limb. The stride frequency corresponds to the number of strides performed per unit of time. The stride frequency is equal to the inverse of stride

duration and it is usually expressed in strides/s or in hertz (Hz).

During a unipodal stance phase, only one limb is in contact with the ground, as in the gallop. One forelimb and hindlimb can be synchronized in two different ways.

1. During a *diagonal stance phase* — for example, at the trot — a hindlimb and the contralateral forelimb are in contact with the ground at the same time. The left diagonal is composed of the left forelimb and right hindlimb; the right diagonal is composed of the right forelimb and left hindlimb.
2. During a *lateral stance phase* — for example, at the pace — the hindlimb and forelimb of the same side are in contact with the ground at the same time.

When the forelimbs and hindlimbs hit the ground non-synchronously during a lateral or diagonal stance phase, the time elapsed between the hindlimb and forelimb contact is called the *advanced placement*. It is positive if the hindlimb hits the ground first. Similarly, the advanced lift-off can be measured between the hooves lifting off.

The stride length corresponds to the distance between two successive hoof placements of the same limb. The *over-reach* or *overtrack* is defined as the distance between the hindlimb imprint and the ipsilateral forelimb imprint. It can be positive or negative if the imprint of the hindlimb is in front of the forelimb imprint (positive) or behind it (negative).

The number of lines defined by the successive hoof imprints on the ground defines the number of tracks. This qualitative information characterizes the transverse motion of the gait. If the horse locomotion is slow and straight, there should be two tracks because the forefeet are exactly in the same line as the ipsilateral hindfeet. In some dressage exercises, such as 'shoulder in', 'haunches in', 'quarters in', 'quarters out', or 'half pass', the horse can use two, three, or four tracks. During the examination of a hindlimb lameness, three or four tracks can be observed and usually the lame hindlimb follows one of the median tracks. The fast trot of a harness trotter usually shows four tracks with the two hindlimbs most abaxial.

Variety of gaits

The main characteristics of the equine gaits are described in Table 2.5.1. Within each gait there exist continuous variations from slow speed with a collected gait to higher speed with an extended gait. Two

types of gait can be distinguished by the symmetry of the limb movement sequence with respect to time and the median plane of the horse:

- *Symmetric gaits*: walk, trot, toelt (paso) and pace
- *Asymmetric gaits*: canter, transverse and rotary gallop.

Several methods have been proposed to classify the gaits according to their temporal characteristics. The continuum of symmetric gaits was described by a diagram proposed by Hildebrand.⁵¹ The stance duration of the hindlimb was plotted against the lateral advanced placement. On the *x* axis, the stance duration of the hindlimb indicates if the gait is walked (no suspension phase) or run (two suspension phases). On the *y* axis, the lateral advanced placement quantifies the asynchrony or the phase lag of the lateral forelimbs and hindlimbs. The two-beat gaits are up and down the diagram and the four-beat gaits are in the middle part of the diagram. Another diagram has been proposed for illustrating the diagonal gaits by plotting the hind stance phase duration against the diagonal advanced placement.⁵²

Another method, based on a series of coupled oscillators, has been proposed to describe and simulate both symmetric and asymmetric gaits. This model also has the advantage of describing the gait transitions and unique gaits like 'aubin' and 'traquenard'. The model uses four coupled oscillators which simulate the cyclical patterns of the four limb movements. By using five methods of coupling the oscillators, it was possible to generate all types of equine gait. This type of functional model can be useful to understand the locomotor control which includes rhythmic pattern generators.

Symmetric gaits

Walk

The walk is a four-beat gait with a large overlap time between stance phases of the limbs. This is the slowest equine gait but probably one of the more complex because of overlap and lag phase variability. During lameness examination, the variability of stride regularity and symmetry measured at the walk was higher than at the trot.⁵³ In dressage horses, the speed of the walk increases from the collected walk (1.37 m/s) to the extended walk (1.82 m/s) and simultaneously there is a small increase in stride frequency.⁵⁴ The change in speed was primarily the result of lengthening of the stride by increasing the overtracking distance. Even in horses trained for dressage, the regular

Table 2.5.1 Gait characteristics

Classification	Gait	Gait variations	Footfall sequence	Rhythm (beats/stride)	Type of symmetry	Speed (m/s)	Stride length (m)	Stride frequency (strides/s)	Limb stance phase (s or % stride)	Suspension phase (s or % stride)
Symmetric gaits	Walk	Collected, medium, extended	RH, RF, LH, LF	4	Right/left bipedal	1.2–1.8	1.5–1.9	0.8–1.1	65–75%	0
	Toelt = Paso		RH, RF, LH, LF	4	Right/left lateral	3.4–5.3	1.7–2.3	2.23–2.36	40–55%	0
	Trot	Piaffe, passage, collected, medium, extended, flying trot	RH–LF, Susp., LH–RF, Susp.	2	Right/left diagonal	2.8–14.2	1.8–5.9	0.9–2.52	26–53%	0–9%
	Pace		RH–RF, Susp., LH–LF, Susp.	2	Right/left lateral	9.1–16.0	4.5–6.3	1.8–2.4	0.130–0.138 s	0.081–0.094 s
Asymmetric gaits	Canter	Collected, medium, extended, disunited	Trail. H, Lead. H–Trail. F, Lead. F, Susp.	3	Asymmetry with a phase lag between limb pairs	2.9–9	1.9–4.6	1.6–2.0	0.28–0.30 s	0–0.013 s
	Gallop	Transverse, rotary	Transverse: Trail. H, Lead. H, Trail.F, Lead.F, Susp.	4	Asymmetry with a phase lag between limb pairs	9–20	4.5–7.2	2.27–2.92	0.085–0.09 s	0.063–0.114 s 16–28%

four-beat rhythm of the footfall was observed in only one of the six horses measured.

Toelt

Icelandic and Paso Fino exhibit a four-beat symmetric lateral gait (Table 2.5.1). This gait can be called 'toelt', 'paso', 'rack', 'foxtrot', or 'slow gait'. The toelt is comfortable for the rider because the amplitude of the dorsoventral displacement is lower than at the trot. The speed ranges between 1.7 and 2.3 m/s and the natural gait transition for toelter is walk-toelt-canter.

Trot

The slow trot is a two-beat symmetric diagonal gait. Among the normal variations of the trot of saddle horses, the speed of the gait increases from collected to extended trot. Passage and piaffe are two dressage exercises derived from collected trot. However, their temporal variables are different, as was shown in the dressage finals at the Olympic Games in Barcelona.⁵² The stride duration is longer (i.e. slower stride frequency) for piaffe (1.08 s) and passage (1.09 s) than for collected trot (0.84 s). For most of the other temporal variables, collected trot and passage were very similar, except for the suspension phase which was shorter in passage. A positive diagonal advanced placement measured at the collected trot was observed in the elite dressage horses.^{52,55} This means that the hindlimb hits the ground about 20–30 ms before the diagonal forelimb.

In harness trotters, the trot is so extended that it can reach a maximum speed of 14.2 m/s with a maximum stride frequency of 2.52 strides/s and a maximum stride length of 5.92 m.⁵⁶ The diagonal sequence is usually affected and this particular gait is named the flying trot. It is a four-beat gait because there is an asynchrony of the impact and/or lift-off of the diagonal.⁵⁷ In most cases the hindlimb touches the ground first (a positive advanced placement). The dissociation at lift-off is greater than at impact.

Various trot irregularities can occur during a harness race and the horse can be disqualified by the gait judges: at the aubin, the forelimbs gallop and the hindlimbs trot, while at the traquenard, the forelimbs trot and the hindlimbs gallop. A trotter is also disqualified if it breaks into a pace or gallop. With increasing speed the stride length increases linearly but the interference between the hindlimb and the lateral forelimb becomes a limiting factor. A large over-reach of the hindlimbs can be performed only if the hindlimbs follow two lateral tracks (track abaxial to the forelimbs) during the swing phase.

Pace

This lateral symmetric gait is used in harness racing, mainly in North America and Australia. The maximum speed can be faster than at the flying trot. It is also a four-beat gait at high speeds with dissociation of lateral symmetry at impact and lift-off. The hindlimb hits the ground before the ipsilateral forelimb. This lateral advanced placement of the hindlimb is about 26–30 ms. In comparison with the flying trot there is less problem with limb interference because the lateral sequence avoids any contact between the ipsilateral limbs. Consequently, there are fewer coordination difficulties and it is easier for the horse to increase stride length. These differences may explain the faster speed records obtained by pacers (9.4–16.0 m/s) than by trotters (11.8–14.2 m/s).

Asymmetric gaits: canter and gallop

Canter and gallop refer to the same gait at increasing speed; the canter is a three-beat gait at slow speed and the gallop is a four-beat gait at a higher speed. At the canter, the diagonal stance phase is synchronized, while at the gallop the footfalls of the diagonal limbs are dissociated. The first hindlimb hits the ground before the diagonal forelimb at the gallop. The gallop is the fastest equine gait and is used by racing horses such as Thoroughbreds and Quarter Horses.

These two gaits are composed of asymmetric movements of the hindlimbs and forelimbs. Because of this asymmetry, each limb is referred to differently; the lead limb is the last one of the limb pairs to leave the ground. The contralateral limb is called the non-lead limb or trailing limb. Consequently, there are two possible symmetric footfall sequences: right lead canter and left lead canter, and similarly, right lead gallop and left lead gallop. In free conditions the horse prefers to canter or gallop on the right lead to go into a right curve. If it is cantering on the right lead before going into a left curve, the horse will probably change the lead limb to maintain balance in the curve.

The lead change is the transition between right lead canter and the left lead canter footfall sequence. It is commonly accomplished by initially having the horse change the order of hindlimb placements and then the forelimb placements. However, in dressage the rider can elicit the canter lead change during the suspension phase. Racing horses change leads eight or more times per mile to avoid excessive muscular fatigue due to asymmetric work of the limbs and also to minimize the centrifugal forces as they accommodate to the curve.⁵⁸

At the gallop, there are two types of footfall sequence called the transverse gallop and the rotary gallop. The transverse gallop is more frequently used by the horse than the rotary gallop but the latter may be used briefly under some circumstances: for example, after a lead change or when muscular fatigue occurs during a racing gallop. A disunited canter occurs with the same footfall sequence as a rotary gallop except that the stance phase of the diagonal is synchronized. It can be observed for a few strides after a bad lead change in dressage or following a jump.

The jump is a unique gallop stride where the airborne phase is a long dissociation of the diagonal. The footfalls of the jump stride are: trail-H, lead-H at take-off and trail-F, lead-F at landing. At take-off, the hindlimb stance phase is more synchronized than in a normal gallop stride to provide a powerful push-off. The footfalls of the forelimbs at landing are not synchronized.⁵⁹ A lead change can take place during the airborne phase and in this case the change of forelimb placement order takes place before those of the hindlimbs. A disunited canter can be observed after the jump if the lead change of the hindlimbs does not occur immediately after the landing phase.

Gait transitions

To increase its velocity, the horse can switch gait from walk to trot and from trot to canter and then extend the canter to gallop. Each gait can also be extended by changing the spatial and temporal characteristics of the stride. From a dynamic point of view, a gait transition can be characterized by a gait change with non-stationary motions of the limbs. Periodicity and rhythm of the limb motions change suddenly. Gait transition is one of the most difficult basic exercises in dressage where specific coordination should be learned during training exercise.

There is little information about gait transitions in the scientific literature. The footfall sequence of various gait transitions has been described by Marey and Lenoble du Teil.^{18,50} One kinematic study described four types of footfall sequence observed in dressage horses during the walk–trot transition.⁶⁰ One accelerometric study described the instantaneous changes of stride frequency and dorsoventral activity during all types of transition recorded in competitions.⁶¹

From an energetic point of view, it appears that each equid has a preferred speed for the trot to gallop transition and this particular speed is related to an optimal metabolic cost of running.⁶² However, another experiment demonstrated that the trot–gallop

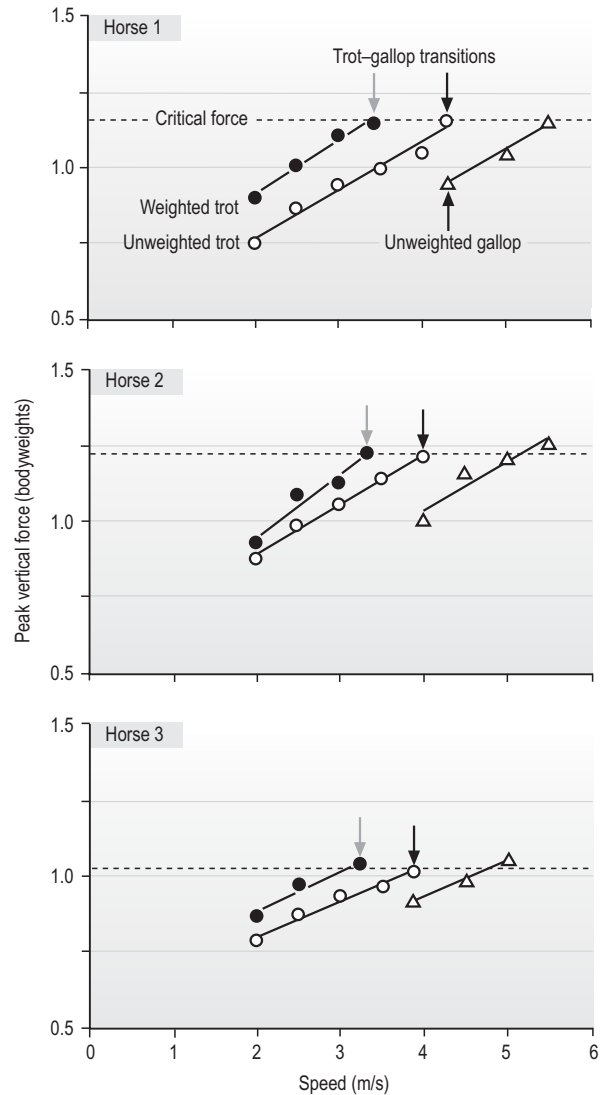


Fig. 2.5.10

Gait transition determinism. When the horses carried weights, they switched to a gallop at a lower speed but essentially the same critical level of forces as when they did not carry weights. By switching to a gallop, the peak ground reaction force was reduced by an average of 14%. The open circles and triangles denote unweighted trotting and galloping, respectively, and the closed circles denote weighted trotting. The plotted values are the average of the peak forces under each of the four limbs. The lines are linear least squares regressions. (Reproduced from Farley & Taylor,⁶³ with permission.)

transition is triggered when the peak of ground reaction force reaches a critical level of about 1–1.25 times the bodyweight (Fig. 2.5.10).⁶³ Carrying additional weight reduced the speed of trot–gallop transition.

Velocity-related changes in stride characteristics

For increasing the speed at a particular gait, the amplitude of the steps becomes larger and the duration of the limb cycle is reduced in order to repeat the limb movements more frequently. Stride frequency (SF) and stride length (SL) are the two main components of gait speed. The mean speed (V) can be estimated by the product of stride frequency by stride length: $V = SF \times SL$. The velocity-related changes in stride parameters have been studied in many horse breeds and disciplines. Stride length increases linearly with the speed of the gait. Stride frequency increases non-linearly and more slowly.^{5,64,65} For a quick increase of running velocity such as that occurring at the start of a gallop race, stride frequency reaches its maximum value first to produce the acceleration, while the maximum stride length slowly reaches its maximum value.⁶⁶

In Thoroughbred race horses, the fatigue effect on stride characteristics increases the overlap time between the lead hindlimb and the non-lead forelimb, the stride duration, and the suspension phase duration.⁶⁷ The compliance of the track surface also can influence the stride parameters when the horse is trotting or galloping at high speed. At the gallop, stride duration tends to be reduced on a harder track surface.³⁸ There is a slight increase in stride duration on wood-fiber tracks in comparison with turf tracks at the same speed. When the rider stimulated the horse with a stick, a reduction in stride length and an increase in stride frequency corresponding to a reduction of the forelimb stance phase duration were observed. However, velocity was not significantly influenced.⁶⁸

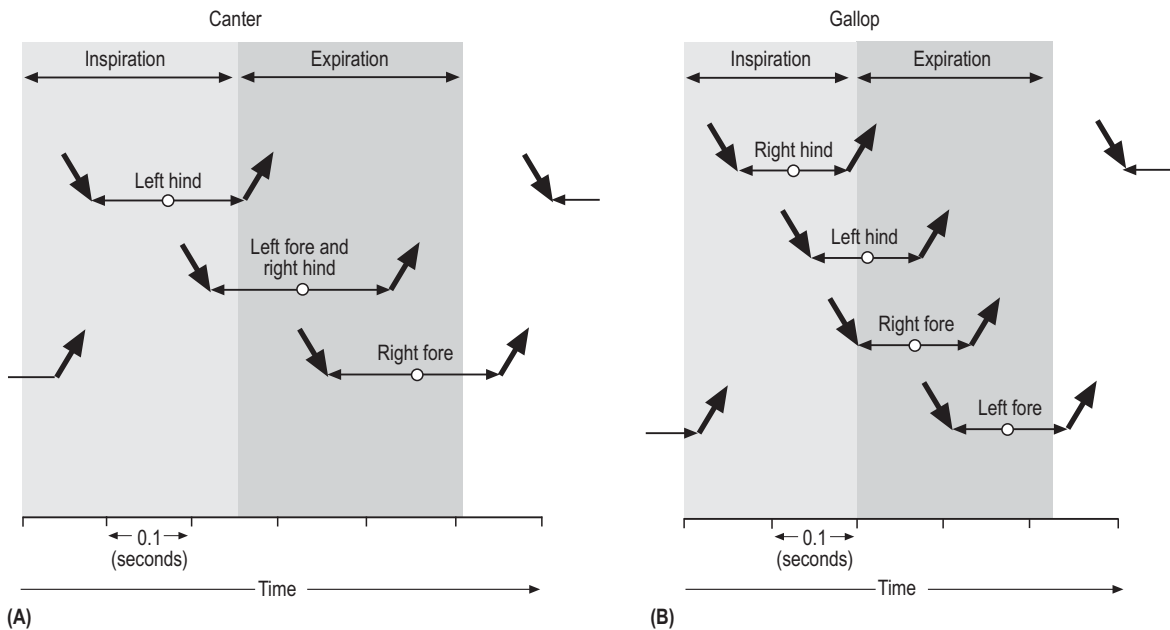
Most of the kinetic and temporal stride variables were influenced by the velocity of the gallop. Stride frequency, stride length, and diagonal dissociation increased linearly with velocity. Velocity was mainly increased by the increase in stride length and secondly by stride frequency. Increase in stride length is mainly explained by the lengthening of the diagonal and airborne phases of the stride.⁶⁵ The overlap duration of the diagonal decreased linearly to about 50 ms as the galloping speed increased.^{58,69} In order to increase gallop stride length, the movements of the hindlimbs and forelimbs are dissociated and length between the footfalls of the diagonal increases. The time elapsed between forelimb mid-stance phases and between hindlimb mid-stance phases is kept constant and independent from velocity.⁶⁵

Respiratory coupling at trot, canter, and gallop

Some relationships have been established between stride parameters and other physiological variables. At the canter and gallop, the respiratory and limb cycle are synchronized. Inspiration starts from the beginning of the suspension phase and ends at the beginning of the non-lead forelimb stance phase. Expiration then occurs during the stance phase of the non-lead and lead forelimbs (Fig. 2.5.11).⁷⁰ Expiration is facilitated by compression of the rib cage during weight bearing of the forelimbs. This functional coupling might be a limiting factor for ventilation at maximal exercise intensity. At the walk, trot, and pace there is not a consistent coupling of locomotion and the respiratory cycle. At a trot, the ratio between locomotor and respiratory frequency ranged between 1 and 3 with respect to the speed, the duration of exercise, and the breed.^{71,72} The same type of low-level coupling was observed at a pace where the ratio between stride and respiratory frequency was 1–1.5.⁷³

Muscle fiber characteristics and locomotion

The relationship between stride parameters and muscle fiber composition was studied in Standardbreds at high speed.⁷⁴ The stride length and frequency were extrapolated at a speed of V_{200} or $V = 9$ m/s. The stride length is positively correlated with the percentages of type I fiber (aerobic slow twitch) and type IIA (aero-anaerobic fast twitch), and negatively correlated with the percentages of fiber type IIB (anaerobic fast twitch). The stride frequency was positively correlated with only the percentage of type IIA fiber. However, in another study the opposite result was found: a negative correlation between the stance duration of young Trotters and the percentage of type IIB fibers.⁷⁵ For race Trotters the force–velocity relationship for skeletal muscles implied in limb protraction and retraction might be an important limiting factor of maximal stride frequency.⁵⁸ In Andalusian horses, there is no significant correlation between the stance duration and the fiber type percentages. However, the diameter of fibers was negatively correlated with stance duration.⁷⁶ The propulsive force during the stance phase might be higher with larger fibers, especially of type I.

**Fig. 2.5.11**

Schematic diagram showing the relationship between the timing of the events of the respiratory cycle and the timing of the events in the cycle of limb movement when a normal horse is cantering (A) or galloping (B), leading with the right forelimb. The distances between arrowheads represent the periods of ground contact of the feet. (Reproduced from Attenburrow,⁷⁰ with permission.)

During a treadmill exercise test, blood lactate concentration and heart rate at high speed seem to be more correlated to stride length than to stride frequency.^{74,77} This finding confirms that the high speed which elicited a cardiac and metabolic response is primarily explained by an increase in stride length. Furthermore, the velocity relating to change of stride frequency is not linear and consequently decreases the coefficient of correlation. In ponies tested on the track, stride frequency was more correlated to blood lactate concentration and heart rate than to stride length, which is more limited in this animal.⁴⁰

Response to training: influence of age and training on locomotor variables

Gait development in foals and yearlings

Gait patterns are influenced by the age of the horse, but little is known about gait development. The rela-

tionship between conformation and stride variables in foals aged 6–8 months has been reported. Speed increases were achieved by longer stride length in heavier foals and higher stride frequency in taller foals.⁵ The elbow, carpal, and fetlock joint angle flexions were the most significant differences between the foals.⁷⁸ The stride and stance duration increased with age but the swing duration and pro-retraction angle were consistent. The joint angle patterns recorded at 4 and 26 months were nearly similar. The good correlations of some of the kinematic parameters measured in foals and adults make it possible to predict the gait quality of adult horses.⁷⁹

Training effects

According to several studies, some of the gait characteristics can also be modified after a training period. However, little is known about the training effect, because few data from longitudinal studies are available. In one study, after 70 days of dressage and jumping training, the stance duration of the hindlimb decreased, its flexion increased, and its maximal pro-

Table 2.5.2 Changes in trot variables with the stage of training in dressage horses

	4 years	5 years	6 years	7 years and older
Stride frequency (strides/s)	1.34 a	1.32 a	1.26 b	1.27 b
Stride regularity (/200)	186 a	185 a	185 a	179 b
Dorsoventral activity (g^2/Hz^2 or W/kg)	7.8	8.3	8.7	10.4
Longitudinal activity (g^2/Hz^2 or W/kg)	1.14	1.62	1.55	2.1
Propulsion acceleration vector (g)	2.3	2.2	1.4	3
Dorsoventral displacement (m)	0.11 a	0.13 b	0.13 b	0.13 b

Means followed by different letters are significantly different at $P < 0.05$.

traction occurred earlier. In addition, the protraction and retraction range of the forelimb decreased and the stride duration was unchanged. After the same period, the horses which had been left in pasture showed other locomotion modifications. They had a longer swing and stride duration and the range of the forelimb movement was larger.⁸⁰

In race horses, the training influence has been investigated in Standardbreds and Thoroughbreds. After 3 years of training, the following changes in the trotting strides were observed: the stride length, the stride duration, and the swing phase increased.⁵⁷ Another study on Standardbreds trained on a treadmill did not show any change in temporal or linear stride variables after 5 months of training. In gallop racing, a stride duration and stride length increase was found.⁶⁷ After 8 weeks of a high-intensity training regime on a treadmill, the stance phase duration of the Thoroughbred gallop stride was reduced by 8–20%.⁸¹

Dressage horses should be trained to improve their coordination, their suppleness, and their gait collection. Collection of the gait means that the forward movement becomes more upward and the stride frequency slows down. A group of dressage horses were tested from 4 to 7 years of age to determine the training-related changes in locomotion variables. The changes of the trot were more pronounced than the changes in the walk and gallop (Table 2.5.2). At the trot, the stride frequency decreases between 5 and 6 years of age. During the same time, walk and gallop stride frequency remains the same. At the trot, dorsoventral displacement increases after the first year of training. The increase of dorsoventral activity with age corresponded to an increase of muscular power and an increase of the dorsoventral displacement with collected gaits. Stride regularity and symmetry increase in early training and then decrease after 6 years of age.

Applications of equine gait analysis

Gait tests for breeding

Early jumping evaluation

Free jumping tests are often used in the performance evaluation of 3-year-old horses. These tests are useful for selection of stallions and mares for breeding, and to select potential performance horses. Gallop, take-off, jump path, and landing characteristics were measured during a free jumping test using the Equimetrix system. Heritabilities of these jump measurements were calculated in French saddle horses to determine the use of breeding criteria (Table 2.5.3). The heritabilities of jumping characteristics are moderate to high and can be used effectively in selection of bloodlines for breeding.

Basic gait characteristics of young dressage horses

Gait and conformation test information is useful in making breeding plans and to identify young horses with good dressage potential more accurately. Gait characteristics may be measured early in a horse's saddle training. Walk and trot may be measured earlier in hand. Several studies were conducted in France and Germany to measure gait and conformation variables in 3-year-old horses presented in a performance test.^{82,83} The relationships between these measurements and the judges' scores were analyzed. The locomotor profile of future dressage horses should have the following gait characteristics:

- *Walk*: large vertical limb displacement and propulsion activity, high regularity

Table 2.5.3 Heritabilities (h^2 and standard errors) of the variables measured during a free jumping test in French saddle horses

Gait variables	h^2 (SE) for walk
<i>Approach strides</i>	
Stride frequency	0.32 (0.19)
Dorsoventral activity	0.50 (0.13)
Longitudinal activity	0.46 (0.20)
<i>Take-off stride</i>	
Sum of the accelerations produced by the forelimbs and hindlimbs	0.47 (0.14)
Power of the take-off exercise	0.40 (0.09)
Take-off impulse duration	0.26 (0.13)
<i>Jump</i>	
Jump duration	0.47 (0.12)
Height of the sternum	0.21 (0.16)
<i>Landing</i>	
Forelimbs deceleration	0.64 (0.13)
Power of the landing exercise	0.64 (0.15)
Speed at landing	0.35 (0.20)

h^2 = heritability; SE = standard error of heritability.

- *Trot*: slow cadence, large vertical displacement, high regularity, large propulsion activity
- *Gallop*: three-beat gallop with high regularity, slow cadence, good propulsion activity.

Horses having these basic gait characteristics obtain better scores in performance tests and they have better performance during the first year of dressage competition.

The 3-year-old German horses evaluated had gait characteristics more advantageously adapted for dressage competition. The results obtained in the best German horses tested could be considered as a reference standard for dressage breeding. Purebred Spanish horses should be considered as a reference standard for collected gaits used in farm work and classical dressage. Specific conformation and gait characteristics in German breeds may explain their higher level of success in dressage competition.⁸⁴ For dressage performance the trot characteristics and its variations are very important because they form the basis of passage and piaffe, movements which are performed at the upper levels of dressage competition. According to the FEI (Fédération Equestre Internationale) standards, the trot should be a two-beat gait with free, active, and regular steps. Regularity and elasticity of the steps and engagement of the hindquarters are important quali-

Table 2.5.4 Comparison of the trot characteristics measured by the Equimetrix system in three groups of breeds used for dressage: German horses (Hannoverian, Westphalian, Oldenburger), purebred Spanish horses, and French saddle horses

Trot characteristics	GER	SF	PRE
Speed (m/s)	3.97 a	4.24 a	3.18 b
Stride length (m)	2.98 a	3.02 a	2.31 b
Stride frequency (strides/s)	1.33 a	1.40 b	1.38 b
Stride symmetry (%)	97	97	96
Stride regularity (/200)	185 a	182 a	174 b
Dorsoventral displacement (10^{-2} cm)	13 a	10 b	10 b
Dorsoventral activity (g^2/Hz or W/kg)	24.33 a	22.01 a	19.76 ab
Percentage of two-beat rhythm (%)	89 ab	90 a	87 b
Propulsion acceleration vector (g)	8.49 a	7.39 b	9.22 a
Propulsion duration (%)	26 a	29 b	29 b
Longitudinal activity (g^2/Hz or W/kg)	2.35 a	1.43 b	2.42 a

GER = Hannoverian + Oldenburger + Westphalian; SF = Selle Français; PRE = Pura Raza Española.

Means followed by different letters are significantly different at $P < 0.05$.

ties of the trot. The same cadence and rhythm should be maintained during trot variations. The trot characteristics of the German horses were consistent with the FEI standards: slow stride frequency, high regularity, large dorsoventral displacement and activity. Activity is considered good when the horse displays elasticity and good propulsion (Table 2.5.4). Spanish horses have shorter stride length, higher stride frequency, and lower dorsoventral displacement and activity than German horses. Spanish horses exhibited elevated movements (large flexion of carpus and tarsus) rather than extended movements of the limbs. It has been shown that purebred Spanish horses have larger elbow and carpus angular range than Dutch Warmblood horses.⁸⁵ Spanish and German groups have high propulsion and longitudinal activity, which should be an advantage for collecting the trot to passage and piaffe. Spanish groups exhibited gaits with lower regularity than the German group but this could be explained by the lower training level of the Spanish horses at this age. Traditionally, purebred Spanish

Table 2.5.5 Heritabilities of the gait variables measured by the Equimetrix system in French saddle horses*

Gait variables	h ² (SE) for walk	h ² (SE) for trot	h ² (SE) for gallop
<i>Stride characteristics</i>			
Speed	0	0.30 (0.14)	NA
Stride length	0	0.29 (0.13)	NA
Stride frequency	0	0.20 (0.15)	0.32 (0.19)
<i>Dorsoventral motion</i>			
Symmetry	0.10 (0.08)	0.12 (0.08)	NA
Regularity	0.10 (0.13)	0.12 (0.09)	NA
Displacement	0.16 (0.12)	0.14 (0.08)	NA
Dorsoventral activity	0.41 (0.12)	0.22 (0.10)	0.50 (0.13)
Gait rhythm	0.29 (0.10)	0.05 (0.07)	NA
<i>Longitudinal motion</i>			
Mean propulsion vector	0.19 (0.07)	0.20 (0.11)	NA
Propulsion duration	0.69 (0.13)	0.38 (0.15)	NA
Longitudinal activity	0	0.44 (0.14)	0.46 (0.20)

* The moderate and high heritabilities are indicated in bold.

h² = heritability; SE = standard error of heritability; NA = non-available data.

horses are broken in at the age of 4 years instead of 2–3 years for German horses.

The mean heritability of dressage performance was rather low ($h^2 = 0.04\text{--}0.27$) as reported by several authors because non-genetic effects like training have a large influence on outcome.^{86–88} Consequently, early selection using gait tests could be more efficient than selection based on performance results. Moderate and high heritabilities have been found for trot and gallop variables such as stride length, stride frequency, dorsoventral displacement, and propulsion variables (Table 2.5.5).⁸³ Some of the gait variables such as stride frequency, dorsoventral and longitudinal activity, which have moderate to high heritability, could be used for improving gait collection ability by genetic selection. Most of the trot variables had a moderate to high heritability (mean heritability $h^2 = 0.24$). The trot characteristics are very important for dressage ability and should be used for early selection at 2 or 3 years of age. Slow stride frequency, high dorsoventral activity, and high propulsion vector and longitudinal activity are the trot characteristics required for performing in dressage. Gallop variables are highly heritable (mean heritability $h^2 = 0.43$) and could also be used for genetic selection. Slow stride frequency and high longitudinal activity are required. Walk heritabilities were rather low (mean heritability $h^2 = 0.15$) except for vertical activity, longitudinal propulsion, and percentage of four-beat walk which were highly heritable. The low heritabilities could be explained because the walk is a complex gait which could be modified by many environmental factors.

Specific locomotion in racing and equestrian sports

Galloping race

Maximum gallop velocity is primarily determined by stride length. An increase in this component is obtained by decreasing the overlap duration of the lead hindlimb and non-lead forelimb stance phase.⁵⁸ The movements of the hindlimbs and forelimbs are dissociated and the length between the footfalls of the diagonal increases. Overlap time of the diagonal decreases linearly down to about 50 ms as galloping speed increases.^{68,69} In poorly performing Thoroughbreds tested on an inclined treadmill (10% slope) at a maximum velocity of 12 m/s, stride length and velocity at maximum heart rate were the variables most highly correlated to running speed.⁸⁹

The racing career of a Thoroughbred is short and the number of racing opportunities is limited. To maximize an individual horse's potential for winning, it should be entered in races appropriate for its racing ability. To help make optimal decisions about entering horses in races, a profile may be developed for each horse while in early training. The profile should include indicators of the animal's speed, endurance, preferred track conditions, and locomotor variables that help determine these characteristics. Locomotor variables of the racing gallop while at maximal speed can only be obtained on a race track because the temporal stride variables should be natural. On a high-speed treadmill maximal speed is limited to 14–15 m/s

and the biomechanics of locomotion are modified by the driven belt.²³ However, the physiological variables such as respiratory variables are more accurately measured on an inclined treadmill at submaximal speed.⁸⁹

A gallop test has been designed to determine the locomotor profile of Thoroughbreds. The gallop test is part of normal 'fast work' training, is easy to perform, and provides locomotor variables to evaluate the racing capacity of Thoroughbreds in training. This test may be used for early evaluation of young horses as soon as they can gallop close to maximal speed on a race track. The results characterize the gallop and could assist the trainer in planning the training and racing program of the horse.

Several gait variables were related to race performance, which was evaluated by the percentage of wins, placings, and the logarithm of average earnings per start. The best performers had a higher stride frequency, stride regularity, and diagonal dissociation. Stride length increase described most of the velocity increase but was negatively correlated with performance. Horses that appeared to be better adapted for short racing distances had higher stride frequency but longer ground contact duration which provided more time for propulsion. It has been found that suspension duration (= stride duration – ground contact duration) decreased at maximal velocities greater than 13–14 m/s.⁶⁹ A quick gallop acceleration at the start of the race is achieved initially by a high rate of stride frequency increase and then by stride lengthening.⁶⁶ During a race, a gallop acceleration is required at the finish to win. Consequently, horses that can increase their stride frequency to the highest value have a better chance to win. A locomotor test performed in harnessed Trotters on a track confirmed that the best race performances were obtained by horses that had the highest maximal stride frequency and a long stride length.⁵⁶

These findings suggest that good race horses are able to trot or gallop at high speed using an optimal stride length and that they can accelerate by increasing their stride frequency in order to finish the race. The best performers reached high stride frequencies of 2.52 strides/s at trot and 2.81 strides/s at the gallop. However, stride length is negatively correlated with performance but stride frequency is positively correlated. Because of pendulum mechanics of the body, maximal stride frequency is dependent on the height of the horse (length of the limbs), weight distribution of the limbs (moment of inertia), elasticity of the limbs and rib cage (module of elasticity), and the percentage

of fast-twitch muscle fibers (forces). Increased propulsive activity produced a much greater velocity. Dorsioventral loading of the limbs was increased at high speed probably because of the large effect of kinetic energy of the body. Dorsioventral displacement of the thorax decreased linearly with velocity and minimized the potential energy changes of the center of gravity which reduced the energy expenditure of locomotion. At the fastest speed, horses reached the 'wheel gallop' by lowering and keeping their center of gravity at the same height during the entire stride, even the suspension phase. The 'wheel gallop' is optimized to produce maximum energy for propulsive work in the longitudinal axis and avoid energy wastage for vertical oscillations of the body. Energy cost of the gallop increases as stride regularity decreases. This may make regularity of the stride one of the limiting factors which determine maximum galloping speed. Regularity was affected by the increase in speed as it has been found in harness Trotters and it is highly correlated with racing performance.³²

Harness racing

In order to measure gait variables during training sessions on a race track, a mobile gait analysis system has been used in harness Trotters.^{41,56} Good Trotters have a short stance phase duration with a longer stance phase in the hindlimbs than in the forelimbs.⁹⁰ A locomotor test performed in race Trotters on a track confirmed that the best race performances were obtained by Trotters that had the highest maximal stride frequency and a long stride length.⁵⁶ These findings suggest that good race Trotters are able to trot at high speed using an optimal stride length and that they can accelerate by increasing their stride frequency in order to finish the race.

Show jumping

During the 1988 Seoul Olympic Games, the kinematics of jumping over a high and wide obstacle (oxer) were analyzed in 29 horses and the relationship to the total penalty score was studied.⁹¹ Fewer total penalties were associated with lower velocities during the jump strides, closer take-off hindlimb placements and closer landing forelimb placements. Another study on elite horses jumping a high vertical fence demonstrated that the push-off produced by the hindlimbs at take-off was associated with the mechanical energy required for clearing the fence.⁹² The action of the forelimbs should be limited to positioning the horse's body into

proper orientation with the jump before the final push-off of the hindlimbs. A more vertical component of the initial velocity was observed in the horses that successfully cleared a wide water jump (4.5 m).⁹³ The angle of the velocity (vector) relative to the horizontal was 15° in a successful jump compared to 12° in an unsuccessful jump, and the vertical component of the velocity was about 0.5 m/s greater in a successful rather than an unsuccessful jump. This initial velocity was generated by the impulse of the hindlimbs and determined the ballistic flight characteristics of the body.

These kinematic findings agree with another study which showed that poor jumpers had a lower acceleration peak of the hindlimb at take-off than good jumpers.⁹⁴ Poor jumpers brake too much with the forelimbs at take-off impulse and the hindlimbs produce an acceleration which is too weak for clearing the fence. This force is one of the main factors affecting a successful jump because it determines the ballistic flight of the center of gravity and also the characteristics of the body rotation over the obstacle during the airborne phase. The moment of inertia and its influence on body rotation were also studied in a group of jumping horses but no consistent relationship with the level of performance was found.⁹⁴ More penalties were recorded for horses that cantered at a low stride frequency (lower or equal to 1.76 strides/s) and suddenly reduced their stride frequency at take-off.

Dressage

In dressage the horse should easily execute complex exercises, gait variations, and gait transitions while maintaining its equilibrium and suppleness. This discipline requires a high level of locomotor control of the horse by the rider, which is achieved progressively through exercise and collecting the gaits. A horse's ability for collection is one of the main limiting factors for dressage because it is impossible to execute the more complex exercises correctly without having attained good basic collection of the gaits. The collected gaits have been extensively described in kinematic studies.^{54,55,95,96}

The trot

Among the normal variations of the trot in saddle horses, the speed of the gait and the stride length increase from collected, to medium and to extended trot. A slow stride frequency, including a long swing phase, is required for good trot quality. The time elapsing between hindlimb contact and diagonal forelimb

contact defines the diagonal advanced placement and should be positive and high at the trot. The horse should place its hindlimbs as far as possible under itself. A large amplitude of vertical displacement of the body during collected trot is desired in the dressage horse. Vertical displacement of the trot is obtained through the storage of elastic strain energy in the fetlock, hock, stifle, and pelvis. For extending the trot, having an inclined scapula (a steeper angle to the scapula), and the amplitude of the elbow joint appeared as important factors. The horses judged to have a good trot should have a large degree of flexion in the elbow and carpal joints at the beginning of the swing phase of the stride. A positive diagonal advanced placement measured at the collected trot was observed in elite dressage horses.^{52,97} In these elite horses the hindlimb contacts the ground about 20–30 ms before the diagonal forelimb. This advanced foot placement could be a means for the hindlimbs to push off earlier during the stance phase.

The degree of collection at the trot could also be measured by the acceleration components of the body. Horses equipped with the Equimetrix system in a dressage test were recorded at all trot variations and passage. With increasing collection at the trot, the vertical component of acceleration increased.⁸³ At the same time, the forward component of acceleration decreased. This indicates that propulsion power in dressage horses is used to increase vertical displacement instead of longitudinal displacement.

The passage and piaffe

Passage and piaffe are two dressage exercises derived from the collected trot but the kinetics of each are quite different. Differences in the temporal variables were demonstrated in kinetic analyses of the dressage finalists at the Olympic Games in Barcelona.⁵² Stride duration is longer (i.e. stride frequency is slower) for piaffe (1.08 s) and passage (1.09 s) than for the collected trot (0.84 s). For most of the other temporal variables, collected trot and passage were very similar except for the suspension phase, which was shorter in passage.

Specific kinetics of passage and piaffe were analyzed on experienced horses using the Equimetrix system. Mean dorsoventral and longitudinal acceleration vectors were measured during the exercise. At the passage, the mean acceleration vector shows that the largest component of propulsion is dorsoventral instead of longitudinal as it is in the extended or working trot. The high collection of passage transforms forward propulsion into upward propulsion. The greatest propulsive activity in passage is from

the forelimbs, while the hindlimbs provide for braking.

At the piaffe, the amplitude of dorsoventral acceleration during the stance phase is lower than at the passage because the amplitude of dorsoventral displacement is reduced. Hindlimbs have greater braking activity to avoid forward movement of the body. The forelimbs have moderate dorsoventral propulsion. The same type of result was observed in a horse at the piaffe with a force plate.⁹⁸ However, at the passage the longitudinal ground reaction force of the hindlimbs was higher than in the forelimbs.

The canter

At slow speed the canter is a three-beat gait. At the canter, the diagonal stance phase is synchronized while at the gallop the footfalls of the diagonal are dissociated. The speed of the canter increases from the collected to working, medium and extended canter by increasing stride length. While stride length increases, stride frequency remains nearly constant.⁹⁵ Stride length increase is due to increase of diagonal step length and distance covered during suspension.

Relationships between the total dressage competition score and canter characteristics in Olympic dressage horses revealed that the best horses were able to extend their canter strides by increasing their stride length without changing their stride frequency.⁹⁹ For three-day event horses at the Olympic games, extended canter stride length and velocity were positively associated with points awarded by judges. However, non-finishers of the event had higher extended canter stride lengths and velocities in the dressage phase than finishers.¹⁰⁰

The lead change is the transition between the footfall sequence of two different canter leads. Normally the hindlimb sequence changes first, then the front limb sequence. In dressage the rider can elicit the canter lead change during the suspension phase. In a bad lead change, the change in forelimb placements occurs before the change of the hindlimbs, resulting in a disunited canter, which may be observed for several strides. The canter during the two and one tempi lead change is slow (3.35–3.95 m/s) with a short stride length (2.08–2.44 m) and a stride frequency of 1.62 strides/s.¹⁰¹ Two tempi changes result in a change in the canter lead every other stride and one tempi change results in canter lead changes on every stride.

During the canter pirouette, the canter footfall sequence is altered. The cadence is reduced from 1.58 stride/s at the collected canter to 1.13 strides/s.⁹⁶ The diagonal support is entirely dissociated and there is no suspension phase.

The walk

In dressage horses, the speed of the walk increases from the collected walk (1.37 m/s) to the extended walk (1.82 m/s) and simultaneously there is a little increase in stride frequency.⁵⁴ The speed change was mainly the result of the stride lengthening by increasing the overtracking distance. Even in horses trained for dressage, the regular four-beat rhythm of the footfall was observed only in one of the six horses evaluated. The stride regularity could be assessed by dorsoventral acceleration recording. Extended walk has a more regular pattern than collected walk where the footfall sequence is slightly disturbed by the actions of the rider. Elite dressage horses usually have a very regular acceleration pattern at the walk. This is a sign of a high level of coordination and stability in the rhythm of footfall sequence.

Gait transitions

A study of transitions from trot to walk showed a positive correlation between a higher level of training and a 'clean' transition without a short intermediate step.⁶⁰ The combination of gait analysis by accelerometric measurement and wavelet analysis allowed quantification of some of the temporal and kinetic characteristics of gait transitions.⁶¹ Transition duration, dorsoventral activity and frequency were specific to each transition. Training improved smoothness of braking deceleration and frequency changes with a longer transition duration.

The transition duration increased significantly with training for trot–walk, canter–halt, and canter–trot transitions. The lengthening of the transition duration allowed a slow decrease of stride frequency (canter–trot transition) and a smooth decrease of vertical activity (canter–halt and trot–halt transition). The rider adapted his technique to locomotion and training level of the horse. By lengthening transition duration, experienced horses could perform a smooth deceleration. In contrast, young horses could not adequately prepare their gait transition and suddenly brake their forward motion, which produced a high peak of deceleration. The amplitude of frequency change during the gait transition decreased with training for all transitions, especially for canter–halt and trot–walk transition.

Three-day eventing

The analysis of the gallop stride characteristics of three-day event horses during the steeplechase of the

Seoul Olympic Games revealed optimal values for successful performance. The optimal stride length should range between 1.85 and 2.05 m while the optimal velocity should range between 13 and 14.3 m/s.¹⁰²

Biomechanics of the hoof and lameness prevention in the athletic horse

Effects of the track

The kinetics of hoof impact is an interesting subject for lameness prevention because a relationship between repeated exposure to shock and the onset of chronic injuries has been established in human medicine¹⁰³

and in animal models.^{104,105} The shoes and the track surface can be designed in order to minimize hoof shock intensity, especially for race and showjumping horses which undergo very large hoof decelerations at high speeds and landing, respectively. Hoof shock and vibration acceleration measurements after the moment of impact on the ground were used for testing the influence of the track surface on shock and vibration intensity of hoof impact at the trot.³⁴ The shock of deceleration can reach 707 m/s² on asphalt and 31 m/s² on sawdust mixed with sand. The subsequent transient vibrations have frequencies between 592 Hz and 41 Hz, respectively. The stiffness of the track surface directly influences these mechanical parameters and should be controlled to minimize vibration

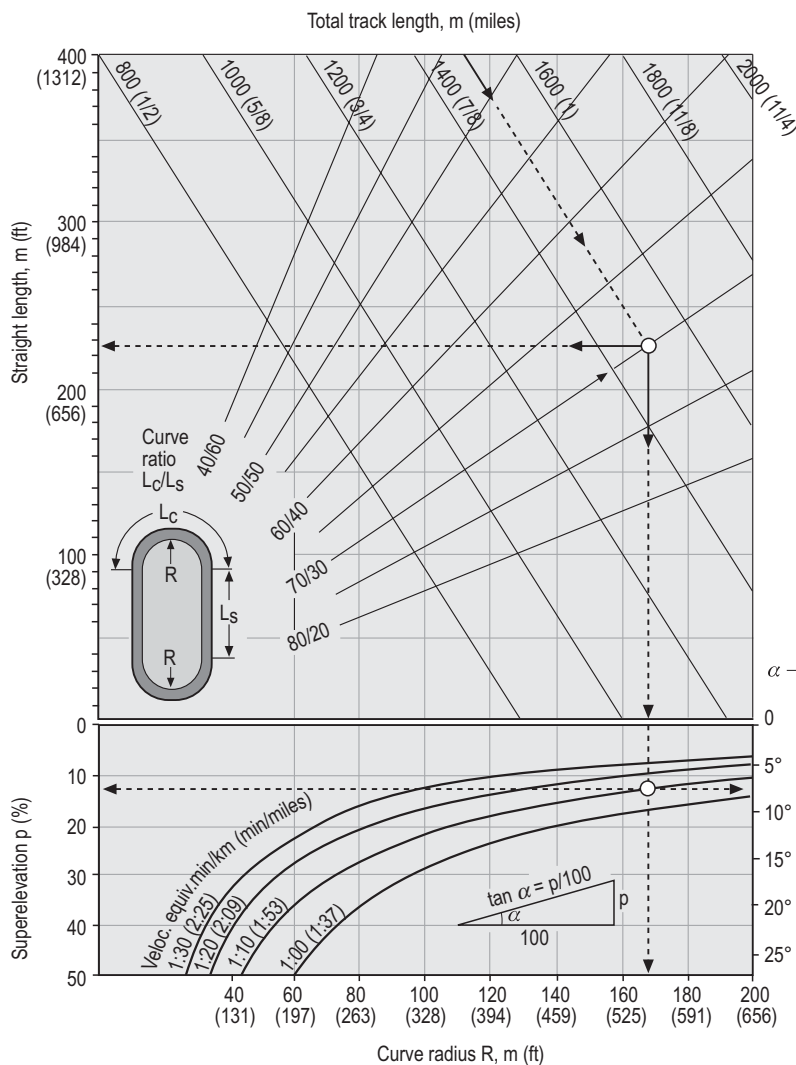


Fig. 2.5.12

Relationship between the geometric characteristics of a track with semicircular curves. Given a track length of 1500 m and a curve ratio (ratio of curve length to straight length) of 70/30, the length of each straight is read as 225 m and the curve radius as 167 m. For a velocity equivalent of 1:10 min/km the superelevation of the curve is read off on the scale as approximately 12.5% or 7°. (Reproduced from Fredricson et al.,¹⁰⁶ with permission.)

damage. In horses, as in human athletes, damping hoof impact with viscoelastic shoes and a soft track surface is useful to prevent overuse injuries of the distal joints.

Improvements in race track designs and surfaces for race horses exemplify the application of research for the betterment of horses and the racing industry. The limb kinematic studies on Standardbreds trotting on various types of racetrack (length, curves) made it possible to propose some recommendations regarding the ideal geometry for racetracks (Fig. 2.5.12).^{106,107} The most important factor affecting comfort is the total length of the track, which determines the curve length and the horses' inclination to avoid any load disequilibrium between the lateral and medial sides of the limbs.

Effects of shoeing

Many shoeing techniques are available but any assumption concerning their effects on hoof biomechanics should be verified experimentally by locomotion analysis studies. The limb kinematics of six sound horses was studied at trot in hand to determine the effect of long toes and acute hoof angles.¹⁰⁸ In this study, no lengthening of the trotting strides was observed after reducing the hoof angle about 10° from

the normal value. However, this hoof angle change resulted in toe-first impact and prolonged breakover, both of which are potentially disadvantageous for athletic performance and may predispose the horse to injury. In another study, the effect of an acute angulation of the hind hooves showed some changes in the limb coordination which could be interesting for reducing limb interference. The hind hoof breakover can be prolonged and the lift-off of the hindlimbs delayed, which could prevent interference with the ipsilateral forelimb.

Hoof shock and vibration acceleration measurements after the moment of impact on the ground were used to investigate the damping capacity of various hoof pads and shoes.³⁵ Compared to the reference steel shoe, shock reduction was higher for light shoes made of a polymer and/or aluminum alloy which had lower stiffness values and density than steel (Fig. 2.5.13). The use of viscoelastic pads contributed to shock reduction and attenuated the high-frequency vibrations up to 75%.

Damping hoof impact with viscoelastic shoes and a soft track surface is useful to prevent orthopedic overuse injuries of the distal joints. The shock damping potential of the track surface is higher than that of the shoes but when the track surface is too hard, the use of damping shoes is highly recommended for horses.

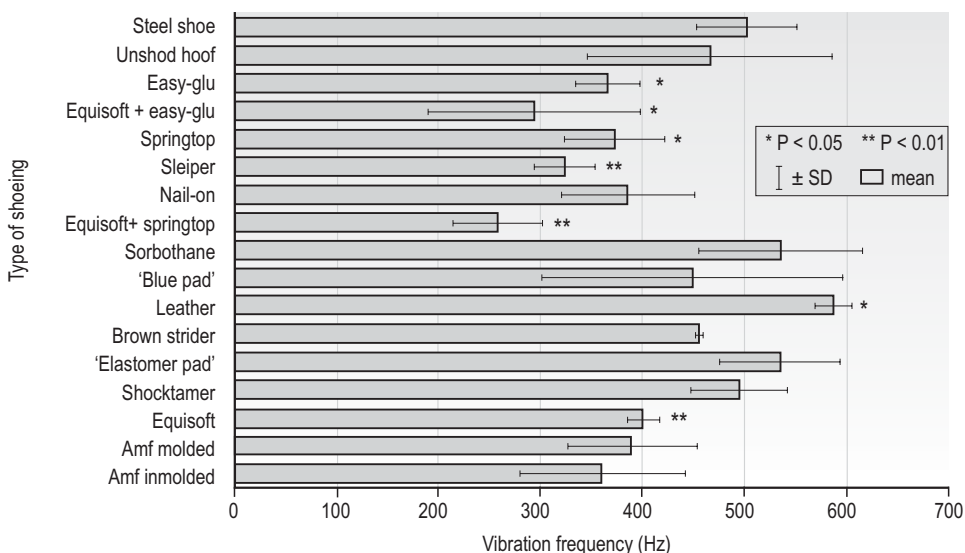


Fig. 2.5.13

Comparison of the hoof vibration recorded by an accelerometer at the trot on an asphalt road with different types of shoeing (shoe and pads). Better damping of the hoof vibrations after impact was observed with light shoes and soft pads. (Reproduced from Benoit et al.,³⁵ with permission.)

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Upper airway function of normal horses during exercise

Susan J. Holcombe & Norm G. Ducharme

The horse is a magnificent athlete that can run in excess of 30 miles per hour. In order to accommodate the tremendous oxygen demand of skeletal muscles during such intense exercise, the horse increases its minute ventilation nearly 50-fold. High airflow rates required to meet this ventilatory demand are created principally by diaphragmatic contraction, which produces driving pressures within the upper airway exceeding -30 cmH₂O. Because the horse is an obligate nasal breather and rarely breathes orally, the horse's upper airway must quickly prepare for these large changes in airflow and pressures by dilating and becoming more rigid (less compliant). Such accommodation is achieved by synchronous and coordinated contraction of upper airway muscles and constriction of capacitance vessels within the mucosa of the upper airway.^{1,2} These remarkable and somewhat unique adaptations of the equine upper airway to exercise are the subject of this chapter.

Obligate nasal breathing

Teleologically, obligate nasal breathing is thought to be advantageous to a fight or flight creature because obligate nasal breathers can graze and masticate while moving air through the nasal passages, maintaining olfaction and the ability to sense predators.³ To accomplish this, the soft palate is tightly apposed to the base of the larynx, such that there is no communication between the oropharynx and the nasopharynx, as exists in people (Fig. 3.1.1A,B). In people, the 'switch point' from nasal to oronasal breathing during exercise is determined by the flow resistance in the nasal airway and the turbulence of the airflow.⁴ Estimated airflow rate associated with the switch from nasal to oronasal breathing is variable and reported to

be between 22 and 44 L/min.⁴⁻⁶ Horses maintain nasal breathing, normally, throughout exercise and rely on capacitance vessel constriction and contraction of upper airway dilating muscles to minimize airflow resistance.¹ Exactly at what level of exercise intensity these factors occur in exercising horses is not known.

Basic upper airway mechanics

The upper airway begins at the nares and includes the nasal passages, the nasopharynx, and oropharynx, guttural pouches, larynx, and trachea. Some segments of the upper airway, such as the larynx, are supported by cartilage, and some portions, such as the nasal passages, are supported by bone. The pharyngeal region is formed principally by skeletal muscle and relies on the contraction of these muscles and muscles of the hyoid apparatus and tongue for stability.

At rest, the average 450 kg horse breathes 12 times per minute with a tidal volume of 5 liters and peak inspiratory flow of 5.09 ± 0.34 L/s, making its resting minute ventilation approximately 60 L/min.⁷ During intense exercise, respiratory frequency increases to 120 breaths per minute, peak inspiratory airflow increases to 75 ± 9.35 L/s, tidal volume increases to 12–15 Ls, and minute ventilation increases to approximately 1400–1800 L/min.⁷ All the upper airway segments are exposed to varying degrees of negative pressure as the diaphragm contracts during inhalation, creating negative driving pressure for airflow to the lungs. These pressures range from -1.9 ± 0.2 cmH₂O during normal tidal breathing to -38.6 ± 3.9 cmH₂O while running at speeds that result in maximal heart rate ($HR_{\max}$).⁸ The ratio of the peak

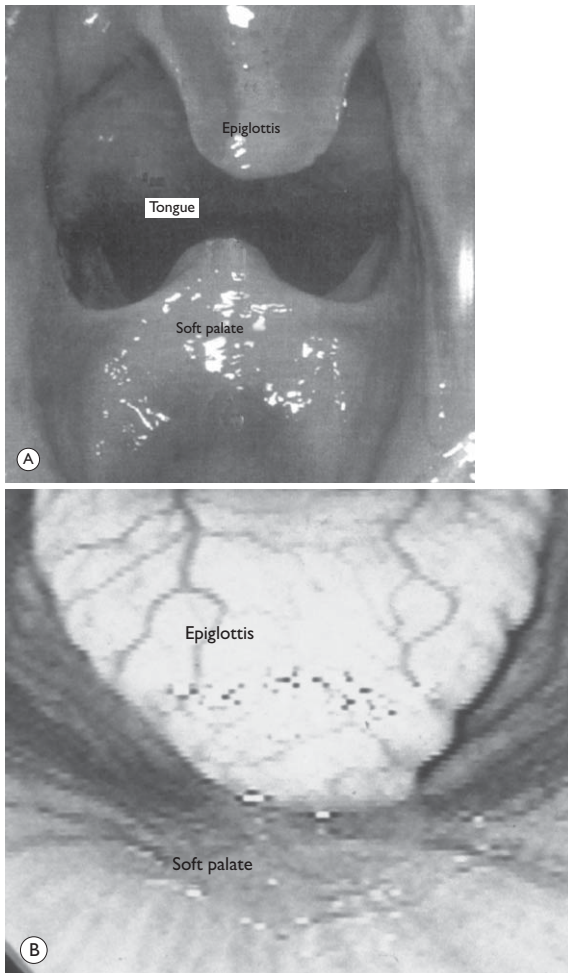


Fig. 3.1.1

(A) Endoscopic image of a human oropharynx and nasopharynx. Notice the space between the epiglottis and the soft palate, which forms the communication between the oropharynx and nasopharynx in people. (B) Endoscopic image of the equine epiglottic and soft palate apposition. There is no communication between the oropharynx and nasopharynx in the horse due to the tight apposition of the soft palate and epiglottis.

pressure that occurs to produce a given peak airflow is the airway impedance, and impedance is a measure of how the airflow is opposed by the airway.⁹ The determinants of airway impedance include resistance, which is dependent on the airway geometry, and the elastance and inertance of the tissues.⁹ The most important component of airway impedance is resistance, which is principally determined by the radius of curvature of the airway or its diameter. Resistance

is defined by the formula $R = (8hl)/\pi r^4$, where h is the viscosity of the air, l is the length of the airway, and r is the radius.¹⁰ The viscosity of air does not change and the length of the airway changes minimally with head and neck flexion and extension. The diameter of the airway, however, does change during inhalation and exhalation. In the resting horse, two-thirds of the total resistance to airflow resides in the upper airway.¹ As the horse inhales, the largest pressure changes occur at the nares and larynx due to narrowing in these areas.¹ During exercise, upper airway resistance increases to 80% of total airway resistance because the tissues of the upper airway tend to collapse dynamically as airway pressures become more negative.¹ Positive pressure tends to dilate the upper airway during exhalation and therefore upper airway resistance to airflow during exhalation is only 50% of total expiratory airway resistance.¹ Static or dynamic obstructive airway disease can result in large changes in airway resistance. For example, if the diameter of the airway decreases by 20% due to a small amount of granulation tissue on the left arytenoid cartilage, airway resistance would double. Therefore, throughout the respiratory cycle, the horse relies on neuromuscular mechanisms to dilate and stabilize the airway during intense exercise in order to accommodate such high flow rates and pressure changes while minimizing resistance to airflow.

Head position

The position of the horse's head and neck does affect upper airway flow mechanics in exercising horses.¹¹ Measurements of airway mechanics made in six horses with the head and neck in neutral, extended, and flexed positions confirmed that head and neck position significantly increases inspiratory impedance.¹¹ The induced obstruction is typical of a dynamic upper airway obstruction because inspiratory values but not expiratory values are altered. Head and neck flexion may cause the airway to be more compliant, because tissues, such as the pharyngeal walls and soft palate, bulge into the airway, or because the nasopharynx shortens with this maneuver. Horses with most types of obstructive upper airway dysfunction show more severe signs of respiratory distress and exercise intolerance while exercising with the head and neck flexed rather than in a neutral or extended position. Posture or head position alters upper airway muscle activity such that head flexion or cervical spine flexion increases genioglossus nerve activity in other

species.^{12,13} Therefore, altered upper airway muscle activity may result from postural changes in head and neck position in exercising horses and these changes may be due to stimulation of local upper airway receptors that sense changes in tissue tension, pressure, and airflow.

Neuromuscular control of upper airway function

During intense exercise multiple stimuli trigger contraction of upper airway dilating muscles, including chemical stimuli such as hypercapnia and hypoxia, limb movement, central locomotor-linked cortical influences, receptors located in the lower airways, and upper airway sensory receptors.^{14–21}

The laryngeal mucosa has an abundant supply of sensory receptors that control a complex pattern of respiratory reflexes that influence the patency of the upper airway and the pattern of breathing.²² These receptors are mechanoreceptors or temperature-sensing receptors and include pressure, flow, and drive receptors that line the mucous membranes and deeper tissues of the nose, nasopharynx, and larynx.²² They receive afferent innervation from branches of the trigeminal, glossopharyngeal, and vagus nerves.^{23,24} Receptors in the nasopharynx are innervated by branches of the glossopharyngeal and trigeminal nerves.²² These receptors are principally tactile receptors and stimulate the gag response, important in airway protection.

Especially relevant to dilation and stability of the upper airway during exercise are the pressure receptors. Pressure receptors account for 60% of the sensory receptors within the laryngeal mucosa in horses, which is similar to other species.^{22,25} These receptors are innervated by the superior laryngeal branches of the vagus nerve.²³ They are stimulated during upper airway obstruction, when large collapsing pressures are produced in the upper airway, and they provide afferent information to the central nervous system, signaling contraction of upper airway muscles to resist dynamic collapse in the upper airway. For example, studies in dogs, cats, rabbits, monkeys, and people have shown that reflex augmentation of muscle contraction by application of negative pressure in the upper airway occurs in the genioglossus and other tongue muscles, muscles of the hyoid apparatus, and the soft palate.^{16,18–21,26,27} In horses, negative pressure stimulates increased electromyographic (EMG)

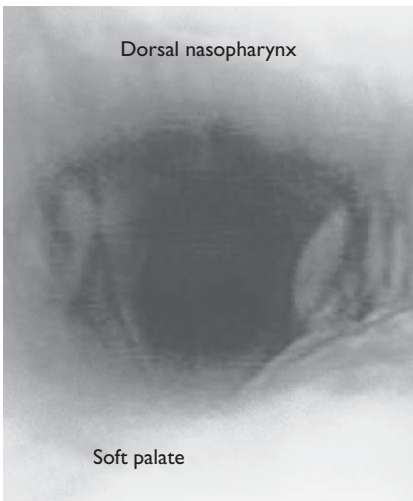
activity in the cricoarytenoideus dorsalis muscle, the primary laryngeal abductor.²⁵ During incremental exercise testing the palatinus, palatopharyngeus, hyoepiglotticus, sternohyoideus and sternothyroideus, geniogyoideus, and genioglossus muscles all had increasing levels of electromyographic activity as treadmill speed increased, and upper airway pressures became more negative.^{28–30}

In species other than horses, it has been noted that the time of application of negative pressure during the breathing cycle is an important variable in determining the magnitude of the response of upper airway muscles. Specifically, upper airway motor neurons are more responsive during early inspiration to pressure changes in the airway than during later stages of inspiration.²⁷ The onset of inspiratory upper airway muscle activity often precedes that of the diaphragm, and is modulated by chemical drive, and mechanical afferent input from the upper airways that is, primarily, vagally mediated.³¹ Many of the upper airway muscles are maximally active during early to mid-inspiration, with a subsequent decrement in activity during the remainder of inspiration. Inspiratory activation of upper airway muscles prior to the diaphragm will dilate or stiffen the upper airway, promoting upper airway patency, prior to the onset of inspiratory airflow and hence produce an early inspiratory stabilization of upper airways.³¹ The degree of negative pressure established in the upper airway will increase the amount of muscular pre-activation.²¹

Topical anesthesia of the luminal surface of the larynx or bilateral superior laryngeal nerve sectioning markedly reduces the response to changes in upper airway pressure and upper airway muscle activity in laboratory species and people.^{24,32} In horses, topical anesthesia of the laryngeal mucosa results in increased inspiratory upper airway and nasopharyngeal impedances, and decreased respiratory frequency and minute ventilation.³³ The dynamic upper airway obstruction was caused by nasopharyngeal collapse due to decreased skeletal muscle support. Following topical anesthesia of the laryngeal mucosa, horses exhibit dorsal displacement of the soft palate and nasopharyngeal collapse during endoscopic examination at rest (Fig. 3.1.2) and varying degrees of nasopharyngeal collapse during incremental treadmill exercise (Fig. 3.1.3).³³ These results suggest that local sensory receptors in the upper airway of horses, as has been shown in laboratory species, contribute to upper airway patency and that disrupting the sensory component of the local reflex that controls contraction of

**Fig. 3.1.2**

Endoscopic image of the nasopharynx while the nares are occluded following topical anesthesia of the laryngeal mucosa. Notice how the nasopharynx collapses, almost forming a sphincter.

**Fig. 3.1.3**

Endoscopic image of the nasopharynx during treadmill exercise (10 m/s) following topical anesthesia of the laryngeal mucosa. Notice how the nasopharynx is collapsing, obstructing the view of the corniculate processes of the arytenoids and the epiglottis.

upper airway muscles can cause dynamic upper respiratory obstruction in horses.

Nasal occlusion

Because negative pressure induces contraction of upper airway muscles, a nasal occlusion test was developed in horses in order to mimic pressure changes that might occur during intense exercise to challenge the upper airway with more negative pressures during resting videoendoscopic examination. Peak tracheal inspiratory pressure during nasal occlusion (-24.9 ± 3 cmH₂O) is not significantly different from peak inspiratory pressure while horses exercise at HR_{max} (-25.6 ± 2.7 cmH₂O), and peak pharyngeal inspiratory pressure is significantly more negative (-28.9 ± 4.9 cmH₂O) during nasal occlusion than while horses exercise at HR_{max} (-17.5 ± 2.1 cmH₂O).³⁴ These data indicate that nasal occlusion in standing horses results in pharyngeal and tracheal inspiratory pressures that equal or exceed those that are generated during exercise at HR_{max}, making it a potentially useful test for evaluating the activity of laryngeal and pharyngeal muscle function. However, anecdotally, horses that exhibit varying degrees of nasopharyngeal collapse at rest frequently have normal airway function during treadmill exercise. As well, horses that readily displace their soft palates at rest frequently displace during treadmill exercise, but the correlation between displacement at rest and during treadmill exercise is not a strong one.

Other sensory receptors within the upper airway include flow and drive receptors. Drive receptors represent 20% of laryngeal sensory receptors in horses.^{22,25} These receptors respond to changes in airway deformation, such as collapse, muscle contraction, and movement of the laryngeal cartilages.²² Flow receptors are temperature-sensing receptors.²² These receptors sense cool air temperatures, which occur as airflow increases. The majority of these receptors are responsive during inspiration, but some (approximately 20%) respond during exhalation, and a small population of the airway sensory receptors is active during both inspiration and expiration.²²

In addition to afferent sensory receptor stimulation in the upper airway, chemical stimuli such as hypercapnia and hypoxia also increase the activity of upper airway dilator muscles.^{16,17} Horses exercising at HR_{max} become hypercarbic (PaCO₂ of 50.2 mmHg) and hypoxemic (PaO₂ of 56.1 mmHg).³⁵ Hypoxia and hypercarbia stimulate inspiratory and expiratory

motor neuron activity.^{36,37} The neural mechanism by which central and peripheral chemoreceptors affect cranial motor neuron activity and signaling upper airway dilating muscles, and the role of vagal afferents in these responses are unknown.

Measurement techniques for upper airway mechanics in exercising horses

Evaluating upper airway function in exercising horses requires a combination of qualitative and quantitative measurement techniques. Videoendoscopic evaluation of the upper airway in exercising horses has proven invaluable in assessing both normal and abnormal airway function. Sometimes combined with visual observations of airway function, upper airway mechanics measurements are made by measuring airway pressures and airflows. Using the data, calculations can be made to determine respiratory frequency, tidal volume, minute ventilation, and impedance. Tidal breathing flow volume loops can be constructed if airflow is quantitatively measured and appropriate computer software is available.³⁸ As well, pressure volume curves can be constructed if both airway pressure and tidal volume are measured, permitting work of breathing to be calculated.¹⁰ Muscle activity can be assessed by measuring the electromyographic activity of muscles.^{16–19} Finally, because many upper airway abnormalities cause the horse to produce unique respiratory-related sounds during exercise, sound analysis can be used to evaluate upper airway function and dysfunction.³⁹

The goal when measuring tracheal pressures in exercising horses is to obtain accurate measurements in a minimally invasive manner. Error in measurements can occur due to the high tracheal airflow velocities in running horses and the presence of the measurement apparatus.⁴⁰ Transcutaneous placement of tracheal catheters minimizes airflow obstruction and provides excellent measurement of tracheal static pressure.^{41,42} However, percutaneous tracheal catheterization, especially repeated catheterization, causes tissue trauma that may be unacceptable. Therefore, nasotracheal catheters are frequently used for measuring tracheal static pressures.⁴⁰ These catheters are constructed using polyethylene tubing with a series of side ports created for pressure measurement.⁴⁰ The catheter is then connected to a differential

pressure transducer (Model DP/45, Validyne, Northridge, CA) and recordings can be made on chart recorders or computers capable of recording respiratory function measurements.

Airflow can be measured qualitatively or quantitatively. Qualitative measurement can be performed using temperature sensors.⁴³ Temperature sensors, such as thermistors, can be placed at the nostril or within the trachea. Respiratory rate and respiratory: stride coupling can be assessed, but quantitation of the airflow is not possible. Face mask systems are used to quantitate airflow measurements.^{43,44} The face mask must cover the horse's nose and mouth, be airtight, and allow for unimpeded nostril dilation. In addition, the mask should be light enough and comfortable for the horse to wear while running. Pneumotachographs are instruments used to measure instantaneous rate of volume flow of respired gas.^{43,44} Briefly, the pneumotachograph is attached to the end of the face mask, and as the horse breathes through the pneumotachograph, it creates a resistance to airflow. The pneumotachograph is calibrated prior to the experiment using a rotameter such that a given pressure, measured using a pressure transducer, is proportional to the airflow. Therefore, the difference in pressure measured across the pneumotachograph is proportional to the airflow rate. A pneumotachograph face mask system imposes added resistance to airflow, and may alter upper airway pressures, respiratory frequency, ventilation, and respiratory pattern in exercising horses.^{45,46} Alternatively, quantitative airflow measurements can be made by ultrasonic flow determination, using ultrasonic pneumotachometers.⁴³ These flow meters impose low resistance and have a high frequency response, but are prone to baseline drift.⁴³

Pressure and airflow can be recorded on chart recorders or computers, allowing calculation of various indices that describe the patterns of the airflow. Peak inspiratory and expiratory pressures and flows are determined by measuring from baseline to the peak of the curve. Impedance (Z) is calculated by dividing peak pressure by peak flow. Tidal volume (V_T) can be determined by measuring the area under the airflow curve during exhalation. Respiratory frequency (f_R) is determined by counting the number of breaths per minute. Minute ventilation ($\dot{V}_E$) is the product of respiratory frequency and tidal volume. Respiratory timing can also be determined by measuring the inspiratory time (T_I) and expiratory time (T_E) for each breath.¹⁰

Tidal breathing flow volume loops can be constructed by plotting airflow and tidal volume. Indices used to describe the loop and the pattern of breathing

include peak inspiratory and expiratory flow, and inspiratory and expiratory flows at various tidal volumes, including 50, 25, and 12.5% of tidal volume.³⁸ Tidal breathing flow volume loop analysis is a very sensitive method for detecting airway obstruction in exercising horses because airflows are so high.³⁸ Pressure–volume curves can also be constructed by plotting pressure and volume. Work of breathing can then be calculated by determining the area under the pressure–volume curve.¹⁰

Sound analysis or spectrum analysis of respiratory sounds has been used to help identify the source of specific airway obstructive diseases and to evaluate the effect of various surgical procedures on airway noise. Respiratory sounds can be recorded using a dynamic, unidirectional microphone positioned in front of the horse's nose.³⁹ Recordings can be made while the horse exercises freely or on a treadmill. The respiratory sounds are then analyzed using a computer-based spectrum analysis program (Spectrogram version 6.0 (shareware), available at: <http://www.monumental.com/rshorne/gram.html>). Spectrograms of horses with laryngeal hemiplegia and dorsal displacement of the soft palate have been described.³⁹

Electromyographic measurements of muscles can be made using unipolar or bipolar fine wire or surface electrodes, if the muscle is superficial. Electromyography provides information about the timing of muscle activity and relative increases and decreases in electrical activity, but does not provide information about muscle lengthening or shortening.⁴⁷ Sonomicrometry can be used to assess muscle lengthening and shortening.⁴⁸

Muscular anatomy and function of the upper airway

The nose

The horse's nose includes the paired external nares, the nasal cavities, and the paranasal sinuses. The nostril has two compartments: a dorsal blind sac called the nasal diverticulum and the ventral part, which is the true nostril.⁴⁹ The alar fold divides the nostril into the dorsal and ventral parts. The nasal cavity is divided in half by the nasal septum and vomer bone. Each nasal cavity has a dorsal and ventral nasal concha, which divide the cavity into dorsal, ventral, middle,

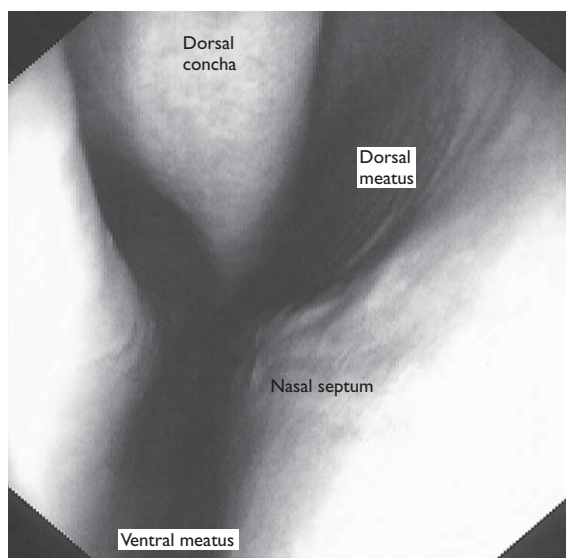


Fig. 3.1.4
Endoscopic image of the concha and turbinates within the nasal passage.

and common meatus (Fig. 3.1.4).⁴⁹ The ethmoid turbinates project from the ethmoid bone in the caudal part of the nasal cavity (Fig. 3.1.5). The nasal valve is the narrowest point in the nasal cavity and, thus, is a major contributor to nasal resistance.¹ This region is caudal to each nostril and immediately rostral to the nasoincisive notch within the dorsal meatus. It is bound medially by the nasal septum, ventrally by the concha, and dorsolaterally by the skin and dorsal conchal fold (Fig. 3.1.6).⁴⁹ Expansion of the nasal valve during exercise occurs by constriction of capacitance vessels and contraction of muscles that pull the skin taut at the dorsal aspect of the notch. The respiratory portion of the nasal cavity has a vascular submucosa, which contains a rich vascular plexus. This plexus is concentrated on the ventral portion of the nasal septum and ventral meatus and is important for warming and humidifying air. The capacitance blood vessels in the airways, especially those lining the nasal mucosa, are innervated by sympathetic, parasympathetic, and peptidergic systems that regulate blood flow.⁵⁰ This extensive vasculature is responsible for warming and humidifying inspired air. When these vessels are dilated, the sinuses and nasal passages become engorged with blood, resulting in airway narrowing and increased airway resistance. Such nasal congestion is a cause of airway obstruction and poor performance in horses with Horner's syndrome.

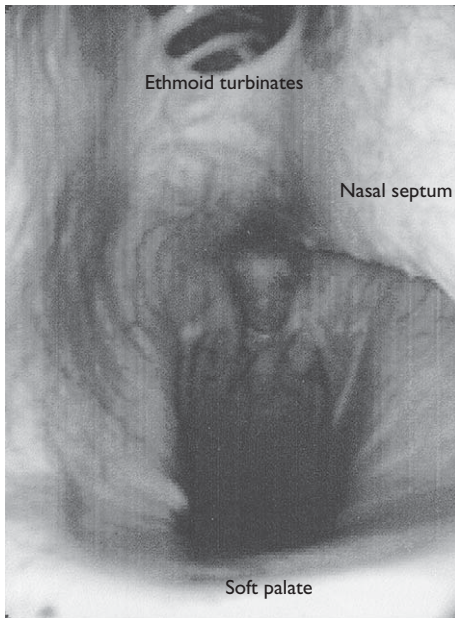


Fig. 3.1.5
Endoscopic image of the ethmoid turbinates and nasopharynx.

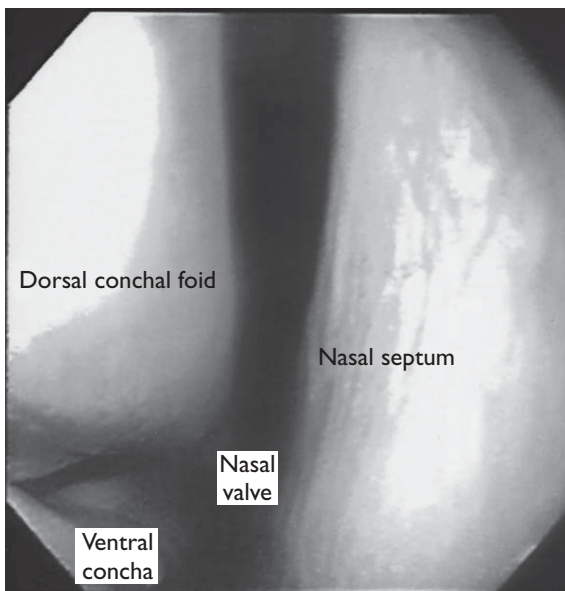


Fig. 3.1.6
Endoscopic image of the nasal valve region.

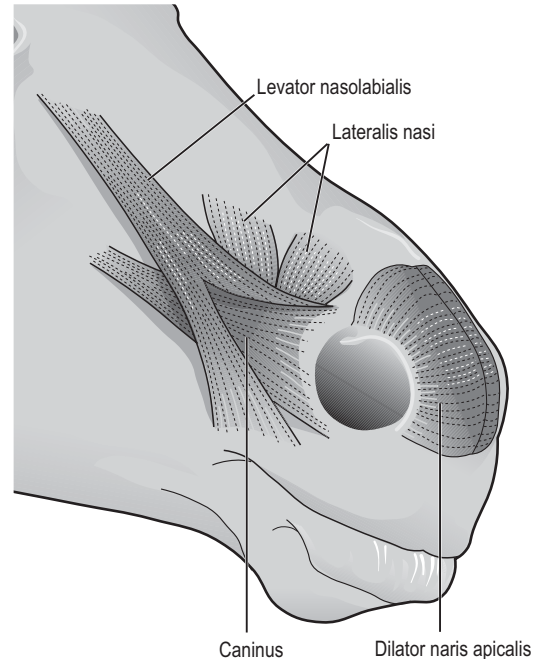


Fig. 3.1.7
Illustration of the dilating muscles of the nares.

During exercise, sympathetic stimulation causes vasoconstriction, increasing airway dimensions and decreasing resistance to airflow.⁵⁰

In obligate nasal breathers such as the horse, increasing nasal patency during exercise is critical to minimizing work of breathing. At rest, the horse's nostril is shaped like a comma, but as the horse increases its respiratory effort the nostril dilates and becomes circular in shape. Horses can actually decrease their nasal resistance during exercise by increasing nasal volume and flaring their nostrils. Nostril dilation is accomplished by the contraction of four different muscles (Fig. 3.1.7).⁴⁹ Contraction of the lateralis nasi dilates the nostril, rotates the conchal cartilages laterally, and expands the nasal vestibule, which forms the floor of the nasal valve.⁴⁹ Other muscles involved in dilatation of the nostrils include the caninus, dilator naris apicales, and levator nasolabialis. Contraction of the caninus or dilator naris lateralis muscle helps expand the lateral aspect of the nostrils.⁴⁹ Dilator naris apicales is an unpaired muscle that lies between the nostrils and aids in dilation of the nostrils.⁴⁹ Levator nasolabialis dilates the nostrils and also elevates the maxillary lip and commissures.⁴⁹ Horses with dysfunction, weakness, or lack of contraction of one or more of these muscles will likely

have dynamic nasal obstruction that may limit performance.

The nasopharynx

The nasopharynx is a musculomembranous unit that functions during breathing, deglutition, and vocalization, and connects the nasal cavity to the larynx. It is attached to the pterygoid, palatine, and hyoid bone, and to the arytenoid, cricoid, and thyroid cartilages by nasopharyngeal muscles that cause pharyngeal dilation and constriction.⁵¹ The nasopharynx is not directly supported by cartilage or bone, yet contraction of these pharyngeal muscles allows the nasopharynx to withstand large changes in intraluminal pressures that occur during tidal breathing at rest and during exercise. Such activation of these muscle groups is synchronous with breathing and this synchronization is coordinated by multiple stimuli.^{17,27} These same muscles are also important during deglutition. This dichotomy of action, contracting the pharyngeal walls during swallowing and dilating the airway during breathing, seems contradictory. But these muscles are uniquely situated to perform both activities, because the pharynx is a conduit for both food and air. Muscles responsible for altering the size and configuration of the nasopharynx include the muscles that alter the shape and position of the tongue, the muscles that control the position of the hyoid apparatus, a constrictor group of muscles located in the dorsal pharynx, and a group of muscles that regulate the position of the soft palate.

Soft palate

The soft palate completely divides the pharynx into nasal and oral compartments in the horse. Because the horse is an obligate nasal breather, it is critically important that the soft palate remains ventral to the epiglottis, except during swallowing, to allow unimpeded nasal breathing. The soft palate extends caudally from the hard palate to the base of the larynx and consists of the oral mucous membrane, which contains ductile openings of the palatine glands, the palatine glands, the palatine aponeurosis, palatinus and palatopharyngeus muscles, and the nasopharyngeal mucous membrane.⁵¹ The caudal free margin of the soft palate continues dorsally, on either side of the larynx, forming the lateral pillars of the soft palate. These pillars unite dorsally, forming the poste-

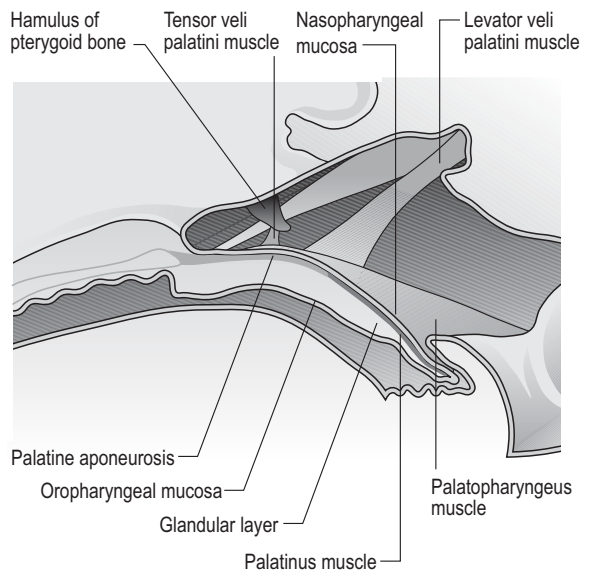


Fig. 3.1.8
Illustration of the muscles of the soft palate.

rior pillar of the soft palate or the palatopharyngeal arch.

The position of the soft palate is determined by the coordinated activity of groups of antagonistic muscles which include the levator veli palatini, tensor veli palatini, palatinus, and palatopharyngeus muscles (Fig. 3.1.8).^{52,53} The levator veli palatini muscle attaches to the petrous part of the temporal bone and the lateral lamina of the guttural pouch. It travels along the lateral wall of the nasopharynx and terminates within the soft palate. A branch of the pharyngeal branch of the vagus nerve innervates this muscle.⁵¹ It acts to elevate the soft palate during swallowing and vocalization. The action of the levator veli palatini muscle can be seen during endoscopic examination of the upper airway when the gag reflex is stimulated (Fig. 3.1.9). A 'sling' forms within the nasopharynx as the nasopharynx contracts into a sphincter.

The tensor veli palatini is a flat, fusiform muscle that, like the levator, attaches to the petrous part of the temporal bone, the pterygoid bones, and the lateral lamina of the guttural pouch.⁵¹ Its tendon is reflected around the hamulus of the pterygoid bone, where it is lubricated by a bursa. The tendon then ramifies in the palatine aponeurosis.⁵¹ It receives motor innervation from the mandibular branch of the trigeminal nerve. Contraction of this muscle tenses the palatine

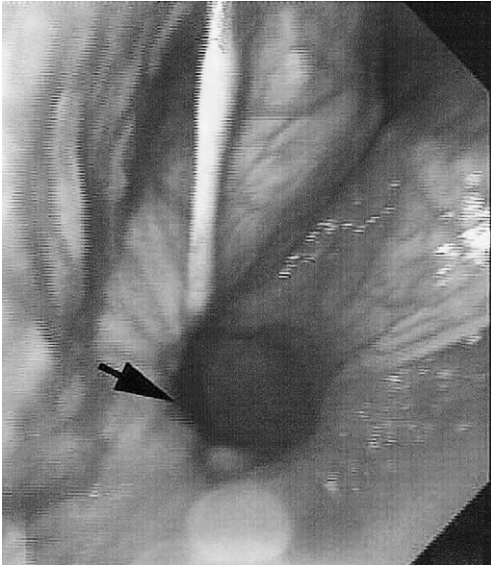


Fig. 3.1.9

Endoscopic image of the nasopharynx during a 'gag' reflex. Notice the 'sling' formed by partial contraction of the levator veli palatini muscles (arrow).

aponeurosis and, therefore, the rostral portion of the soft palate, and depresses this portion of the soft palate toward the tongue.^{51–53} Contraction of the tensor veli palatini muscle also aids in opening the pharyngeal opening of the guttural pouch.⁵⁴ Bilateral transection of the tendon of the tensor veli palatini muscle in horses causes instability of the rostral portion of the soft palate resulting in inspiratory obstruction during intense exercise (Fig. 3.1.10A–C).⁵⁵ The rostral portion of the soft palate is more compliant and its action dependent on airway pressures, such that during inspiration the rostral portion of the soft palate billows dorsally in the airway and during expiration it is depressed toward the tongue by the positive pressure within the airway.⁵⁵

The palatinus muscle (uvula retractor muscle) consists of two fusiform muscles that lie on either side of midline of the soft palate, beneath the nasopharyngeal mucosa, extending caudally from the hard palate.⁵¹ The muscles attach to the caudal aspect of the palatine aponeurosis and terminate near the caudal free margin of the soft palate. A small muscle bundle arising from the lateral aspect of each muscle continues a short distance caudodorsally into the palatopharyngeal arch.⁵¹ It receives motor innervation from a branch of the pharyngeal branch of the vagus nerve.⁵¹

Contraction of the palatinus muscle shortens the soft palate.^{51–53}

The palatopharyngeus muscle originates from the palatine aponeurosis and the lateral border of the palatinus muscle.⁵¹ It travels caudally along the lateral wall of the nasopharynx to the pharyngeal raphe, forming part of the superior constrictor muscle group. A branch of the pharyngeal branch of the vagus nerve innervates it.⁵¹ Contraction of this muscle shortens the soft palate and draws the larynx and esophagus toward the root of the tongue.

Contraction of both the palatinus and palatopharyngeus muscles shortens the soft palate and depresses the caudal portion toward the tongue.^{52,53,56} Both the palatinus and palatopharyngeus muscles receive efferent motor innervation from the pharyngeal branch of the vagus nerve.⁵¹ This nerve branches from the parent vagus nerve at the level of the cranial cervical ganglion and courses cranioventrally along the medial wall of the guttural pouch to the dorsal wall of the pharynx where it ramifies in the pharyngeal plexus. Bilateral local anesthesia of the pharyngeal branches of the vagus nerve induced persistent dorsal displacement of the soft palate and dysphasia in horses.⁵⁷ Horses can become dysphasic, with or without persistent soft palate dysfunction, following guttural pouch lavage with caustic solutions, guttural pouch empyema, trauma, or mycosis.^{58,59} Based on this information, there was convincing evidence to suggest that dysfunction of the neuromuscular group, including the pharyngeal branch of the vagus nerve, palatinus and palatopharyngeus muscles, might be involved in the pathogenesis of intermittent dorsal displacement of the soft palate in exercising horses.

Electromyographic measurements of the palatinus and palatopharyngeus muscles in normal horses exercising on a treadmill showed that these muscles are active, they are synchronous with respiration, and their activity increases as exercise intensity and inspiratory pressures increase (Fig. 3.1.11).⁶⁰ Phasic expiratory activity of the palatinus muscles increases $310 \pm 67\%$, whereas phasic expiratory activity of the palatopharyngeus muscles increases $120 \pm 30\%$ as the treadmill speed increases from 6 m/s to 13 m/s (or until exhaustion).⁶⁰ Palatinus muscle EMG activity is diminished in horses with dorsal displacement of the soft palate and does not significantly increase as treadmill speed increases.⁶⁰

The palatinus muscle is composed of principally type IIA fast-twitch fibers (5–25% type I and 75–95% type IIA) with darkly staining mitochondria, which suggests that these fibers have increased endurance

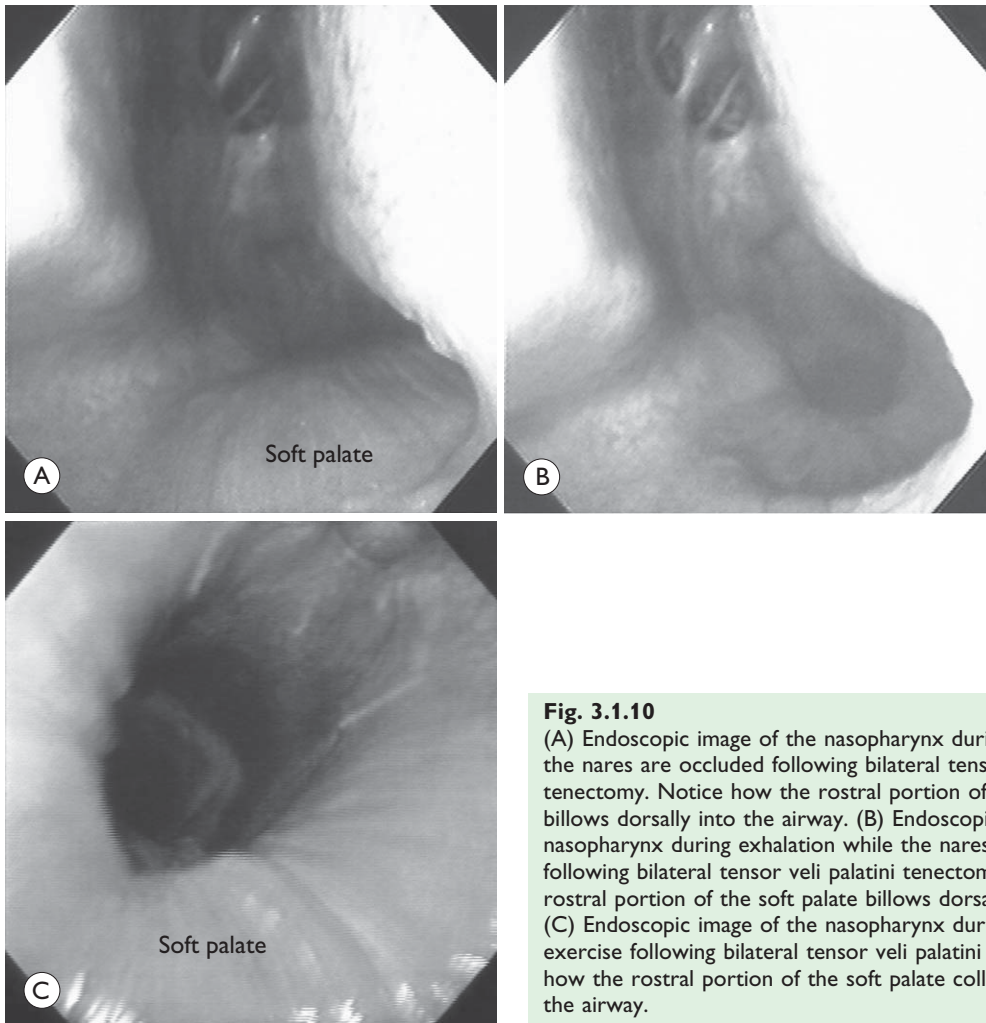


Fig. 3.1.10

(A) Endoscopic image of the nasopharynx during inhalation while the nares are occluded following bilateral tensor veli palatini tenectomy. Notice how the rostral portion of the soft palate billows dorsally into the airway. (B) Endoscopic image of the nasopharynx during exhalation while the nares are occluded following bilateral tensor veli palatini tenectomy. Notice how the rostral portion of the soft palate billows dorsally into the airway. (C) Endoscopic image of the nasopharynx during treadmill exercise following bilateral tensor veli palatini tenectomy. Notice how the rostral portion of the soft palate collapses dorsally into the airway.

relative to most skeletal muscle fast-twitch fibers.⁶¹ The palatopharyngeus muscles are also principally composed of type IIA fibers (10–25% type I and 75–90% type IIA fibers).⁶¹ Pathologic abnormalities are also observed in the palatinus muscle of horses with intermittent dorsal displacement of the soft palate (DDSP). These abnormalities are consistent with chronic denervation and include fiber type grouping, mild atrophy, moth-eaten fibers, and target fibers (Fig. 3.1.12A–D).⁶¹

Muscles of the hyoid apparatus

The hyoid apparatus in horses consists of an assembly of bony rods, some of which articulate together.⁶² Several muscles are attached to this apparatus, and

the contraction of these muscles alters the shape and position of the apparatus, which in turn changes the position and shape of the larynx and nasopharynx.^{63,64} The hyoid apparatus consists of the paired stylohyoid, epihyoid, ceratohyoid, and thyrohyoid bones, and the central basihyoid bone (Fig. 3.1.13). The stylohyoid bone articulates with the petrous part of the temporal bone, allowing the stylohyoid bones to move cranial to caudal, in a pendulous manner. The ceratohyoid bone attaches to the distal end of the stylohyoid bone (by way of the epihyoid bone), and movement at this articulation lengthens the stylohyoid–ceratohyoid unit (Fig. 3.1.14). The base or root of the tongue is attached to the lingual process of the basihyoid bone. The tongue is located on the floor of the mouth between the rami of the mandible. The base of the tongue is

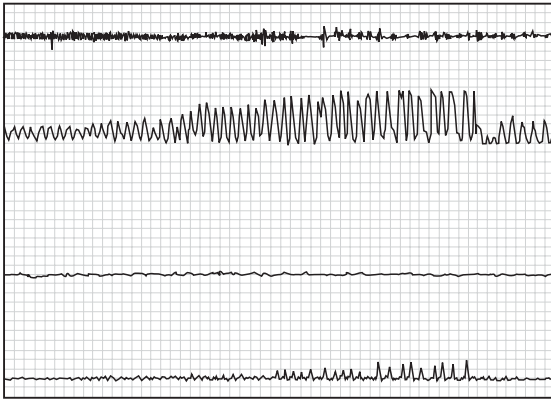


Fig. 3.1.11

Raw and moving time average electromyographic activity tracings of the palatinus and palatopharyngeus muscles during treadmill exercise and during recovery.

attached to the hyoid apparatus, soft palate, and pharynx.⁵¹ Folds of mucous membrane pass dorsally on either side of the base of the tongue to form the palatoglossal arches, which attach the tongue to the soft palate.⁵¹ The genioglossus, hyoglossus, and styloglossus muscles are extrinsic muscles of the tongue that, in part, control the position and function of the tongue and provide attachments to the hyoid apparatus.⁵¹

The genioglossus is a fan-shaped muscle that lies within and parallel to the median plane of the tongue.⁵¹ The genioglossus muscle originates from the medial surface of the mandible, just caudal to the symphysis, and is innervated by the medial branch of the hypoglossal nerve.⁵¹ A large tendon runs throughout the muscle. Muscle fibers radiate rostrally toward the tip of the tongue, dorsally, and distally toward the root of the tongue. The hyoglossus is a flat wide muscle that lies in the lateral portion of the root of the tongue.⁵¹

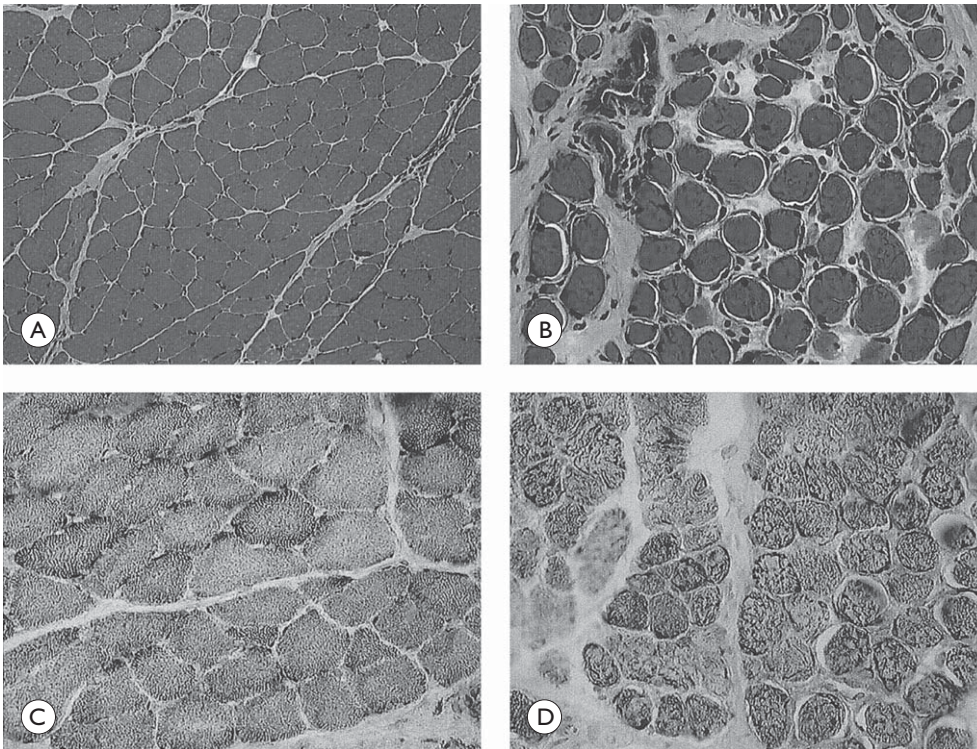


Fig. 3.1.12

(A) H&E stained section of the palatinus muscle from a normal horse. (B) H&E stained section of the palatinus muscle from a horse with intermittent dorsal displacement of the soft palate. Notice the increased amount of connective tissue (light gray) and tissue degeneration. (C) NADH stained section of the palatinus muscle from a normal horse. (D) NADH stained section of the palatinus muscle from a horse with intermittent dorsal displacement of the soft palate. Notice the moth-eaten fibers and areas of increased amounts of connective tissue.

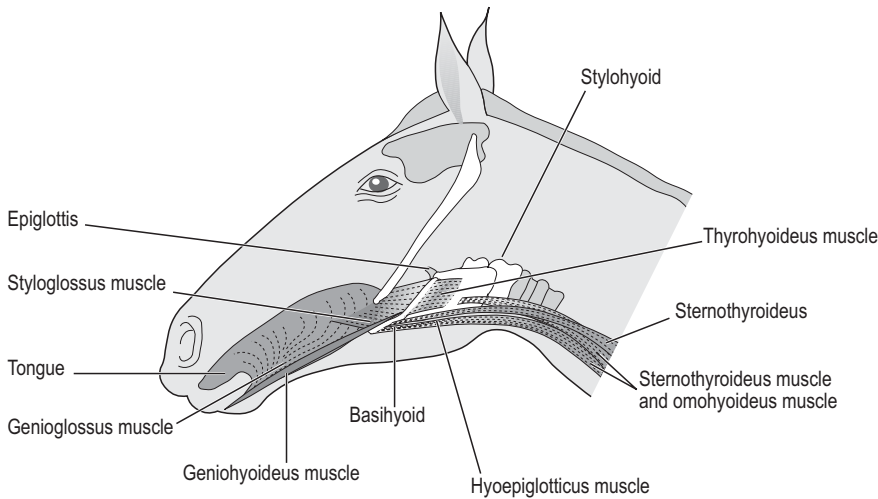
**Fig. 3.1.13**

Illustration of some of the muscles that control nasopharyngeal function.

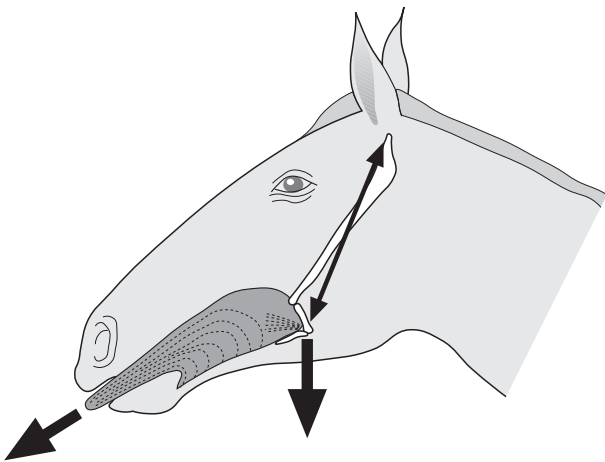
**Fig. 3.1.14**

Illustration showing the effect of various muscles on hyoid length and position.

The hyoglossus originates from the lateral aspect of the basihyoid bone and from portions of the stylohyoid and thyrohyoid bones and is innervated by the lateral branch of the hypoglossal nerve.⁵¹ The styloglossus muscle originates at the distal lateral aspect of the stylohyoid bone and travels the length of the tongue, along its lateral aspect.⁵¹ Near the tip of the tongue the paired muscle meets and ramifies with fibers of intrinsic tongue muscles. Styloglossus contraction retracts the tongue. Contraction of the genioglossus muscle protracts the tongue and pulls the basihyoid bone ros-

trally. Genioglossus also acts with the hyoglossus muscle to depress and retract the tongue. Hyoglossus and genioglossus activity is synchronous with respiration and activity of these muscles correlates well with increases in pharyngeal airway size during breathing.⁶³⁻⁶⁸ Hypoxia, hypercapnia, and airway occlusion caused parallel increases in electrical activity of the protrudor and retractor muscle of the tongue, consistently inducing net retraction and depression of the tongue, improved airflow function, and enhanced pharyngeal stability.^{64,65} Therefore, it seems that tongue depression may be the critical force needed to dilate and stabilize the nasopharynx.

Many horses perform or race with their tongues tied to the mandible or out of the mouth to stop the horse from getting the tongue over the bit, and in an attempt to improve performance, decrease airway noise, and improve airway function. Tying the tongue out of the horse's mouth does not influence the position of the hyoid apparatus or dimensions of the nasopharynx in anesthetized horses (Fig. 3.1.15A,B).^{69,70} In addition, application of a tongue-tie does not alter airway mechanics in normal, exercising horses, suggesting that application of a tongue-tie does not improve upper airway function or alter position of the hyoid apparatus in normal horses.^{69,70} The passive action of pulling the tongue out of the horse's mouth is very different from active muscle contraction. Also, the tongue-tie may cause protrusion of the tongue but not depression of the tongue, and depression of the tongue may indeed be the critical action of the

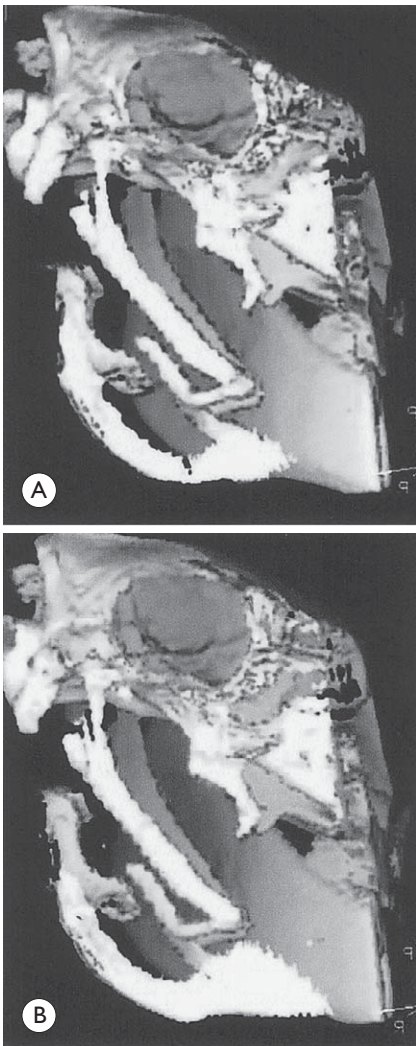


Fig. 3.1.15

(A) Computed tomographic image of the hyoid apparatus of a horse with its tongue tied out of its mouth under general anesthesia. (B) Computed tomographic image of the hyoid apparatus of a horse under general anesthesia with the tongue in a neutral position.

extrinsic tongue muscles that creates upper airway stability and dilation.

Other muscles that attach to the hyoid apparatus include the geniohyoideus, sternohyoideus and sternothyroideus, omohyoideus, and thyrohyoideus. The geniohyoideus muscle is a fusiform, paired muscle that lies on the ventral surface of the tongue.⁷¹ The geniohyoideus originates from the medial surface of the mandible (near the origin of the genioglossus)

caudal to the symphysis and inserts on the basihyoid bone. The hypoglossal nerve innervates it, and its action is thought to move the hyoid bone rostrally.⁷¹ The omohyoideus, sternohyoideus, and sternothyroideus muscles are accessory respiratory muscles that insert on the manubrium and extend cranially. The sternothyroideus inserts on the caudal abaxial aspect of the thyroid cartilage, and the sternohyoideus inserts on the basihyoid bone and the lingual process of the hyoid apparatus. Contraction of these muscles results in caudal traction of the hyoid apparatus and larynx, resulting in dilation of the upper airway.⁷¹ These muscles are innervated by branches of the first and second cervical nerves.⁷¹ Both the sternothyroideus and sternohyoideus muscles are sometimes transected as palliative therapy for horses with dorsal displacement of the soft palate. Following myectomy, translaryngeal and tracheal inspiratory pressures and resistance measurements increase, suggesting that these muscles may act to dilate and stabilize the nasopharynx in normal horses.⁷² The effects of myectomy on airway mechanics in horses with upper airway obstructive disease are yet unknown. The omohyoideus muscles originate on the subscapular fascia near the shoulder joint and also insert on the basihyoid bone and the lingual process of the hyoid apparatus. Contraction of these muscles results in caudal traction of the hyoid apparatus and tongue movement other than retraction.⁷³ The omohyoideus muscles are innervated by branches of the first and second cervical nerves. The omohyoideus muscles have also been transected for treatment of soft palate displacement in conjunction with the sternohyoideus and sternothyroideus muscles but no experimental data exist to investigate the result of omohyoid transection alone or in conjunction with the sternohyoideus and sternothyroideus muscles in horses during exercise.⁷⁴

The thyrohyoideus is a flat rectangular muscle attached to the lateral surface of the thyroid cartilage lamina that inserts on the caudal part of the thyrohyoid bone.⁷¹ It is innervated by the hypoglossal nerve and moves the hyoid bone caudally or the larynx rostrally and dorsally.⁷¹ In studies evaluating the electromyographic activity of some 'extrinsic' nasopharyngeal muscles during exercise, Ducharme and co-workers observed decreased thyrohyoideus muscle activity prior to soft palate displacement in one horse. Investigations by Tsukroff et al.⁷⁵ reveal that transection of a combination of the following muscles results in dorsal displacement of the soft palate in horses: thyrohyoideus, omohyoideus, sternohyoideus and

hyoepiglotticus muscles.⁷⁵ The displacement observed was associated with a more caudal positioning of the basihyoid bone. In subsequent studies thyrohyoid muscle resection caused intermittent dorsal displacement of the soft palate in exercising horses.⁷⁶ As well, thyrohyoid muscle prosthesis, created by placing a suture through the basihyoid bone and the thyroid cartilage, alleviates dorsal displacement of the soft palate.⁷⁶ These data clearly suggest that thyrohyoid muscle dysfunction is the likely etiology of intermittent dorsal displacement of the soft palate in horses.

Dorsal pharyngeal constrictors

The action of the dorsal pharyngeal constricting muscles and the stylopharyngeus muscle is responsible for stiffening and dilating the nasopharynx.^{77–80} The inferior pharyngeal constrictor (thyropharyngeus muscle), middle pharyngeal constrictor (hyopharyngeus muscle), and superior pharyngeal constrictor (palatopharyngeus and pterygopharyngeus muscles) form the dorsal and caudolateral pharyngeal walls.^{78,79} Contraction and shortening of these muscles forms a sphincter, moving the food bolus caudal into the esophagus during swallowing. During breathing, these muscles have tonic and phasic expiratory activities, which help to support the nasopharynx.^{79,80} The major dilating muscle of the dorsal nasopharynx is the stylopharyngeus muscle.⁷⁹ This muscle originates on the axial aspect of the distal portion of the stylohyoid bone and courses rostroventrally to ramify in the wall of the dorsal nasopharynx, by passing between the pterygopharyngeus and palatopharyngeus muscles (Fig. 3.1.16A,B). Contraction of the stylopharyngeus muscles pulls the pharyngeal wall dorsally, to receive the bolus during swallowing.⁸¹ In a similar manner, during breathing, contraction of the stylopharyngeus muscle pulls the nasopharyngeal wall dorsally, thereby supporting the dorsal wall of the nasopharynx and preventing dynamic collapse of this area during inspiration.⁸² The glossopharyngeal nerve provides motor innervation to the stylopharyngeus muscle.⁸¹ Bilateral glossopharyngeal nerve anesthesia produces stylopharyngeus muscle dysfunction, dorsal pharyngeal collapse during both nasal occlusion and exercise, and airway obstruction in horses (Fig. 3.1.17A,B).⁸³ Therefore, the stylopharyngeus muscle is an important nasopharyngeal dilating muscle in horses and dysfunction of this muscle may be implicated in clinical cases of dorsal nasopharyngeal collapse.⁸³

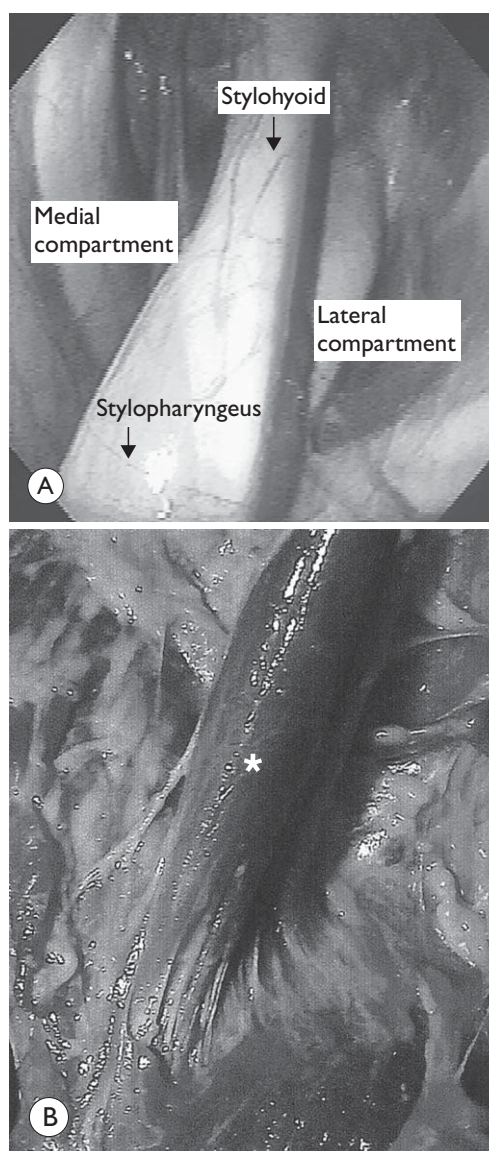
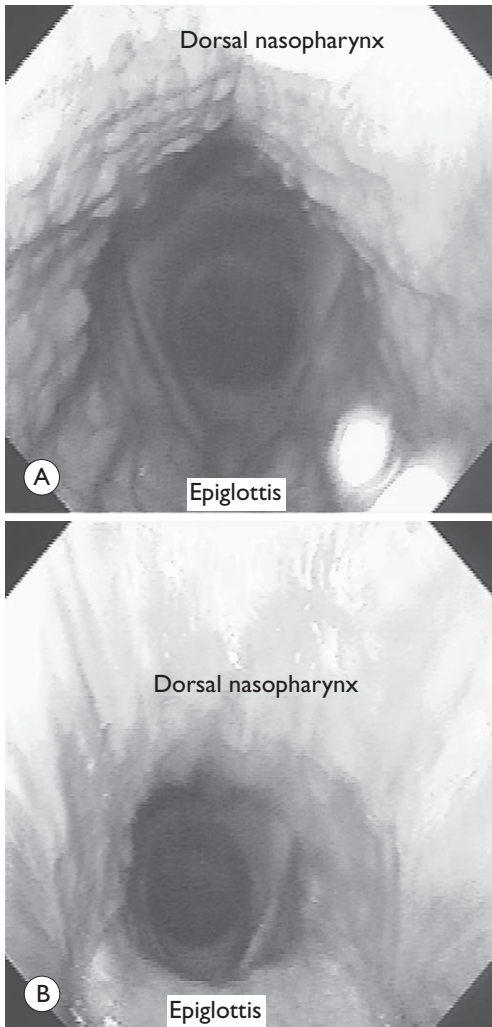


Fig. 3.1.16

(A) Endoscopic image of the origin of the stylopharyngeus muscle on the axial aspect of the stylohyoid bone within the guttural pouch. (B) Post-mortem dissected specimen showing the stylopharyngeus muscle inserting between the pterygopharyngeus and palatopharyngeus muscles.

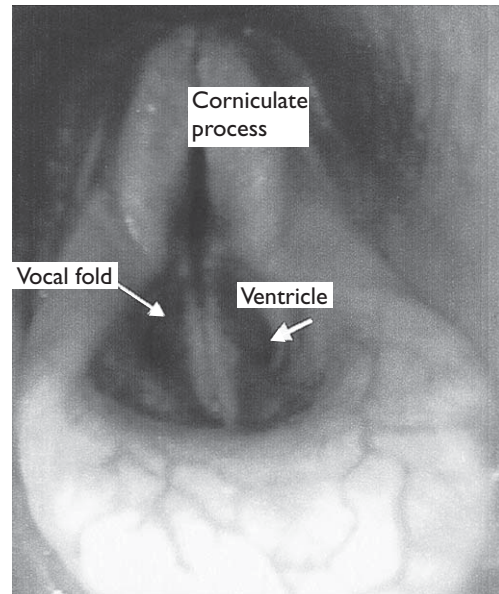
The larynx

The larynx forms the communicating channel between the pharynx and the trachea and functions during breathing, vocalization, and deglutition. The larynx is

**Fig. 3.1.17**

(A) Endoscopic image of the nasopharynx following bilateral glossopharyngeal anesthesia with the nares occluded. Notice how the dorsal nasopharynx collapses. (B). Endoscopic image of the nasopharynx following bilateral glossopharyngeal nerve anesthesia during treadmill exercise (12 m/s). Notice how the dorsal nasopharynx collapses, obstructing the view of the corniculate processes.

composed of cartilage and muscle and is covered with a mucous membrane. The laryngeal cartilages include the cricoid, thyroid, and epiglottic cartilages, which are unpaired, and the arytenoid cartilages, which are paired.⁸⁴ The cricoid cartilage is shaped like a signet ring and is positioned rostral to the first tracheal ring

**Fig. 3.1.18**

Endoscopic image of the larynx during adduction.

and connected to the trachea by the cricotracheal membrane. The thyroid cartilage is the largest of the laryngeal cartilages and is situated just rostral to the cricoid cartilage.⁸⁴ The arytenoid cartilages form the dorsal border of the rima glottidis. They are triangular in shape with a dorsal muscular process, which serves as the origin for the cricoarytenoideus dorsalis muscle, a ventral vocal process serving as the attachment of the vocal ligament, and the rostral apex which forms the corniculate process.⁸⁴ The arytenoid cartilages are positioned on either side of the cricoid cartilage and are connected to it by the cricoarytenoid articulations. The articulation is a diarthrodial joint that allows the arytenoid cartilage to rotate dorsolaterally during abduction and axially during adduction.⁸⁴ The mucous membrane covering the epiglottic cartilage reflects off the lateral border of the epiglottis and blends with the mucous membrane covering the corniculate processes of the arytenoid cartilages, forming the aryepiglottic folds. The mucous membrane covers the vocal ligament, forms the vocal folds, and lines the lateral ventricles, forming the laryngeal saccules (Fig. 3.1.18). These saccules are 2.5 cm deep with a capacity of 5 to 6 mL. They extend between the medial surface of the thyroid cartilage and the ventricularis and vocalis muscles.

The intrinsic laryngeal muscles produce changes in caliber of the rima glottidis by abducting and adducting the corniculate processes of the arytenoid cartilages and the vocal folds and hence altering airway resistance. These actions are accomplished by the contractions of the intrinsic laryngeal muscles. The cricoarytenoideus dorsalis is the principal abductor muscle that widens the laryngeal aperture by abducting the corniculate process of the arytenoid cartilage and tensing the vocal folds. The thyroarytenoideus, arytenoideus transversus, and the cricoarytenoideus lateralis muscles adduct the corniculate processes of the arytenoid cartilages, narrowing the rima glottidis and protecting the lower airway during swallowing.⁸⁴ The cricothyroideus muscle receives efferent motor innervation from the external branch of the superior laryngeal nerve, a branch of the vagus nerve, while all other intrinsic laryngeal muscles receive motor innervation from the recurrent laryngeal nerve, which is also a branch of the vagus nerve.⁸⁴ Crushing or transection of the left recurrent laryngeal nerve, or perineural anesthesia of the left recurrent laryngeal nerve, results in grade IV laryngeal hemiplegia in horses.⁸⁵ However, following experimental crush of the left recurrent laryngeal nerve in ponies, reinnervation of some intrinsic laryngeal muscles is evident.⁸⁵ In ponies, recovery of movement of the vocal folds occurred between 2.5 and 8 months, following recurrent laryngeal nerve crush.⁸⁵ Electromyographic examinations of the laryngeal muscles and microscopic evaluation of the muscles and the recurrent laryngeal nerve reveal that return of function is due to reinnervation.⁸⁵ At times, there is evidence of aberrant reinnervation in abductor and adductor muscles.⁸⁵

The epiglottis is principally composed of elastic cartilage and rests on the dorsal surface of the body of the thyroid cartilage and is held there by the thyroepiglottic ligaments. The position of the epiglottis is controlled by the position of the larynx, and hyoid apparatus, and by contraction of the hyoepiglotticus muscle, which is the only muscle that attaches to the epiglottis.⁸⁴ The hyoepiglotticus is a bilobed extrinsic laryngeal muscle that originates on the basihyoid bone in horses, and inserts on the ventral body of the epiglottis.⁸⁴ In horses, contraction of the hyoepiglotticus muscle pulls the epiglottis toward the basihyoid bone, depressing it against the soft palate and enlarging the airway.²⁸ The hyoepiglotticus muscle has respiratory-related EMG activity in horses that increases with exercise intensity and breathing effort.²⁸ Furthermore, electrical stimulation of the hyoepiglotticus muscle

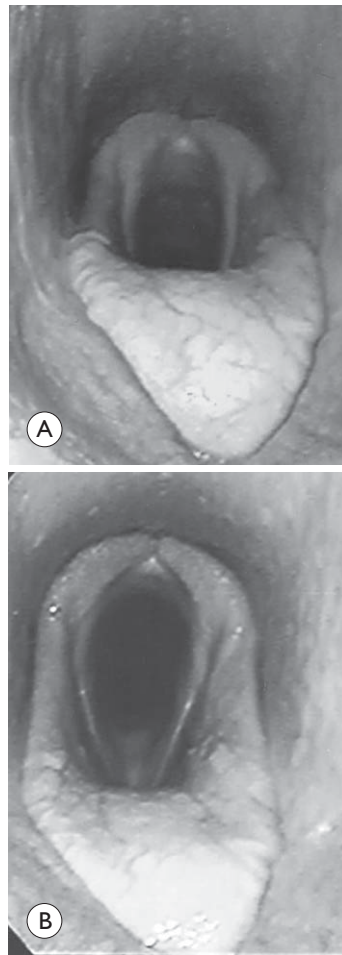


Fig. 3.1.19

(A) Endoscopic image of the larynx. (B) Endoscopic image of the same larynx during electrical stimulation of the hyoepiglotticus muscle.

depresses the epiglottis ventrally against the soft palate, changing the conformation of the epiglottis in some horses (Fig. 3.1.19A,B).²⁸ The hyoepiglotticus muscle is likely an upper airway dilating muscle, which functions to enlarge the airway, thereby decreasing airway resistance in exercising horses. In addition to dilating the aditus laryngis, contraction of the hyoepiglotticus muscle stabilizes the epiglottis during inspiration, preventing its prolapse through the rima glottidis. Retroversion of the epiglottis is described clinically in exercising horses and can be created experimentally by anesthesia of the hypoglossal nerves.^{86,87} Blockade of these nerves creates hyoepiglotticus dysfunction, and dysfunction of other

hyoid muscles including geniohyoideus and genio-glossus, and suggests that the clinical problem may be due to paresis of the hyoepiglotticus muscle or other muscles involved in controlling the position of the basihyoid. Active control of epiglottis position by the hyoepiglotticus muscle apparently stabilizes the epiglottis, and vigorous recruitment of the muscle activity during inhalation dilates the airway and maintains the nasal breathing route in horses during intense exercise. Conformational changes in the epiglottis that occur during exercise, respiratory stimulation, sedation, or nasal occlusion may not be abnormal, but may be the result of normal activity of the hyoepiglotticus muscle.

Guttural pouches

Physiology

The guttural pouch, or diverticulum of the auditory tube, is unique to the horse and other Perissodactyla. Each pouch has a volume of 300–500 mL and communicates with the nasopharynx through the pharyngeal opening of the auditory tube. The guttural pouch is lined with a thin mucous membrane composed of pseudostratified, ciliated epithelium interspersed with goblet cells.⁸⁸ Mucous glands and lymphatic nodules are found deep to the epithelial layer. Various immunoglobulin isotypes, including IgG, IgM, and IgA, have been detected in the guttural pouch mucosa, submucosa, and lymph nodules, suggesting that the guttural pouch has phylactic ability.⁸⁹

Recently, investigators determined that the equine guttural pouches function during selective brain-cooling to maintain blood carried by the internal carotid arteries at a temperature below the core body temperature during hyperthermia, induced by exercise.⁸⁸ Blood is supplied to the brain principally by the internal carotid arteries, with contributions from the cerebral and occipital arteries. The extracranial portion of the internal carotid artery travels through the medial compartment of the guttural pouch. The temperature of the air within the guttural pouch was fairly constant ($37.5 \pm 0.05^\circ\text{C}$) during exercise, and was responsible for cooling the blood within the internal carotid artery by 2°C .⁸⁸ The heat transfer from the internal carotid artery to the guttural pouch was minimal at rest but became more efficient with exer-

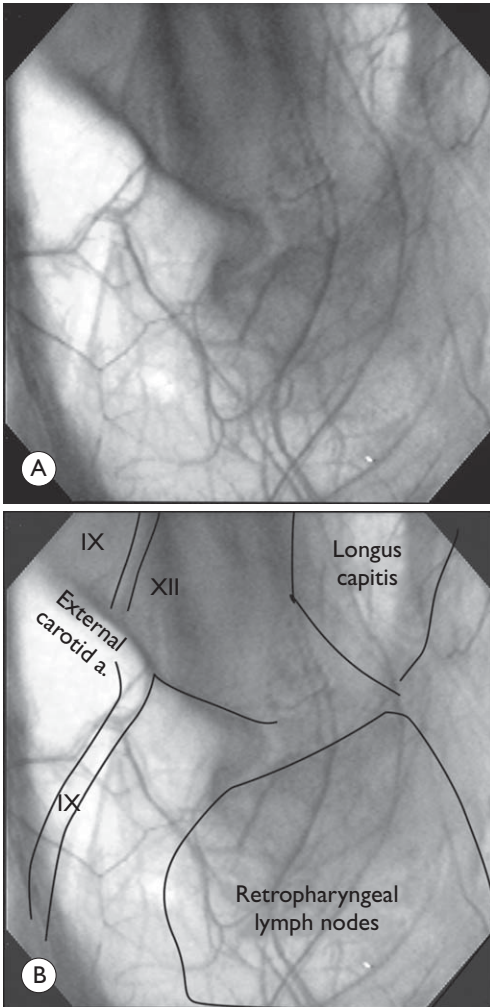
cise.⁸⁸ Therefore, the function of the guttural pouches in the horse seems to be to cool the brain during periods of hyperthermia.⁸⁹

Because of the position of the nasopharyngeal openings of the guttural pouches, changes in nasopharyngeal pressures during inspiration and expiration also affect pressures within the guttural pouches.⁹⁰ When airflow through the nasopharynx is 0 L/s the pressure within the guttural pouches is negative, similar to measurements made in the middle ear of humans.⁹⁰ After swallowing, the nasopharyngeal aperture opens and pressures within the guttural pouches equilibrate with the nasopharynx. Both at rest and during exercise the guttural pouch static pressures are similar to nasopharyngeal pressures, but slightly out of phase with the respiratory cycle.⁹⁰ Movement of the head, chewing, snorting, or swallowing causes changes in pressures simultaneously in both guttural pouches. Therefore, the elevated compliance of the guttural pouch makes it susceptible to pressure changes in the nasopharynx associated with airflow but also with head movement.⁹⁰

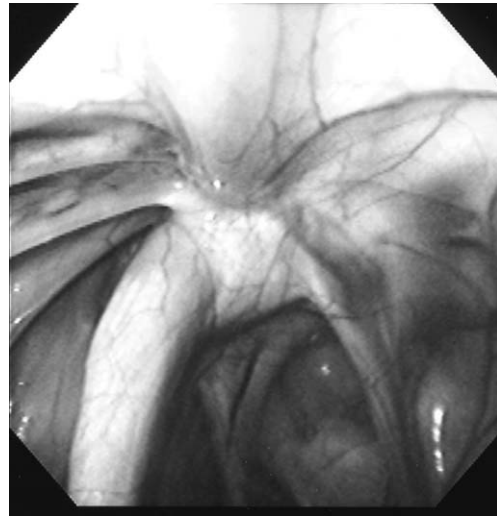
Anatomy

The openings of the guttural pouches are within the nasopharynx. The floor of the pouches forms the dorsal aspect of the nasopharynx, and the caudal extent of the guttural pouch is at the level of the parotid salivary glands. The guttural pouches are bordered dorsally by the base of the skull and the atlas, ventrally by the nasopharynx and rostral esophagus, medially by the longus capitis muscle, the rectus capitis ventralis muscle and the median septum, and laterally by many blood vessels and muscles, including the digastric muscles. Retropharyngeal lymph nodes can be identified beneath the guttural pouch membrane on the floor of the medial compartment (Fig. 3.1.20).

The guttural pouch is divided into medial and lateral compartments by the stylohyoid bone. The caudal portion of the stylohyoid bone articulates with the petrous temporal bone at the base of the skull (Fig. 3.1.21). Cranial nerves VII (facial) and VIII (vestibulocochlear) exit the cranium near this articulation. The opening from the middle ear into the guttural pouch is with the dorsolateral compartment, near the articulation of the stylohyoid and petrous temporal bones. The medial compartment is approximately twice as large as the lateral compartment, and cranial nerves IX

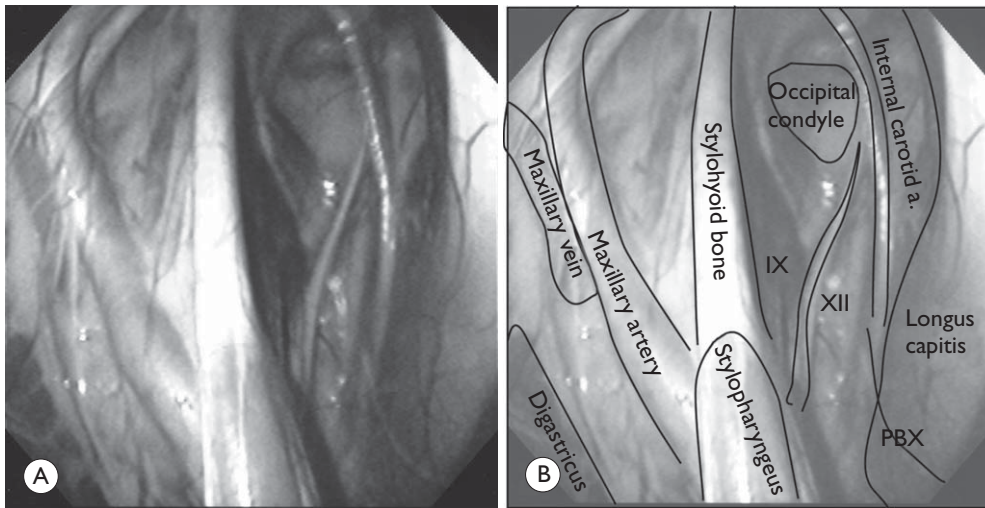
**Fig. 3.1.20**

(A) Endoscopic image of the ventral medial compartment of the right guttural pouch. (B) Endoscopic image of the ventral medial compartment of the right guttural pouch with figure labels. Notice how IX (glossopharyngeal nerve) and XII (hypoglossal nerve) course together, caudal to the external carotid artery. XII dives deep to the external carotid artery, and IX travels across the external carotid artery, rostrally in the guttural pouch. a, artery.

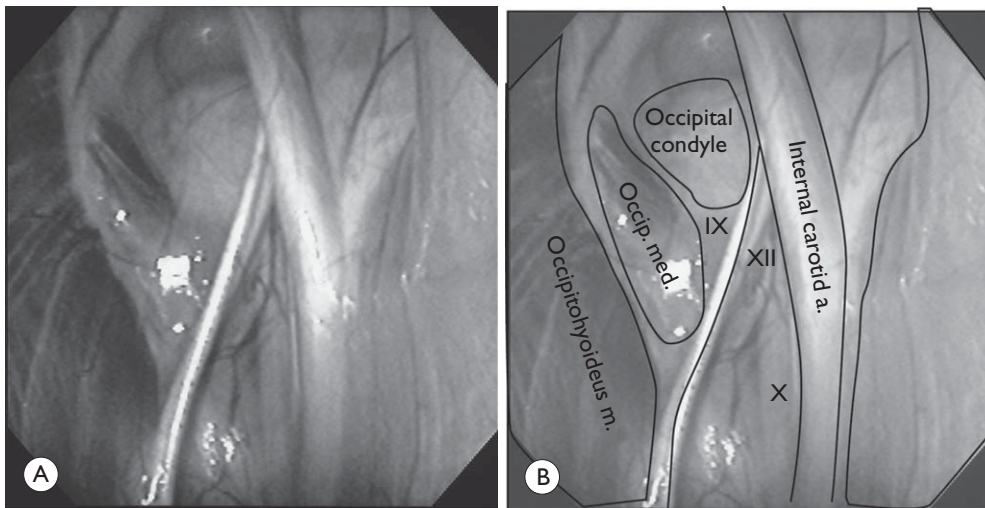
**Fig. 3.1.21**

Endoscopic image of the dorsal portion of the right guttural pouch and stylohyoid/petrous temporal bone articulation.

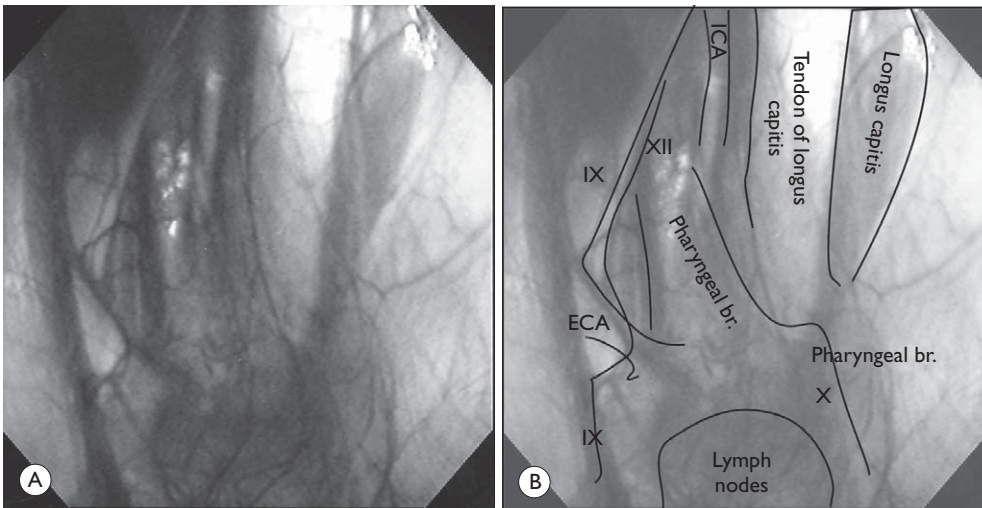
(glossopharyngeal), X (vagus), XI (accessory), and XII (hypoglossal), the sympathetic trunk, the cranial cervical ganglion, and the internal carotid artery lie beneath the lining of the medial compartment (Figs 3.1.22 and 3.1.23). Cranial nerve X, the sympathetic trunk, and the cranial cervical ganglion are closely associated with the internal carotid artery. The pharyngeal branch of X is given off near the cranial cervical ganglion and can be seen as it runs rostroventrally in the guttural pouch toward the wall of the dorsal pharynx, where it ramifies with the pharyngeal branch of IX in the pharyngeal plexus. The pharyngeal branch of IX can be identified as it runs rostrally across the ventral aspect of the stylohyoid bone (Fig. 3.1.24). The maxillary artery is a continuation of the external carotid artery, beyond the origin of the superficial temporal artery, and runs dorsally in the lateral compartment of the guttural pouch. The maxillary vein can be seen lateral to and slightly deep to the external carotid artery. A portion of the digastric muscle can be seen along the ventrolateral wall of the lateral compartment (Fig. 3.1.25). The levator veli palatini and the tensor veli palatini muscles arise, partially, from the lateral lamina of the auditory tube, and can be seen within the pouch, as they pass rostrally and ventrally along the lateral lamina, the wall of the nasopharynx, to the soft palate. At the most caudal aspect of the guttural pouch, the occipital condyle can be seen.

**Fig. 3.1.22**

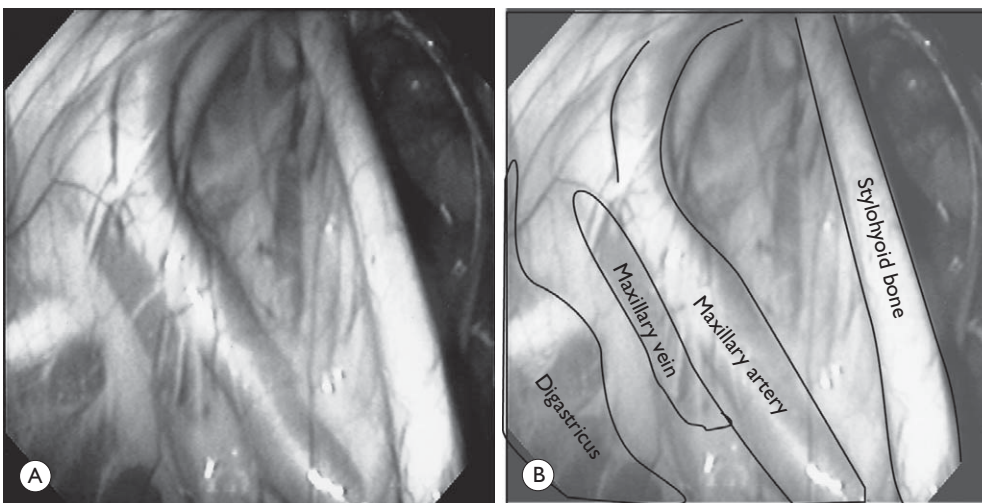
(A) Endoscopic image of the medial and lateral compartments of the right guttural pouch. Medial is the right side of the image. (B) Endoscopic image of the medial and lateral compartments of the guttural pouch with figure labels. IX, glossopharyngeal nerve; XII, hypoglossal nerve; PBX, pharyngeal branch of the vagus nerves. a, artery.

**Fig. 3.1.23**

(A) Medial compartment of the right guttural pouch. (B) Medial compartment of the right guttural pouch with figure labels. IX, glossopharyngeal nerve; XII, hypoglossal nerve; X, vagus nerve; a, artery; m, muscle; Occip. med., occipitohyoideus, medial muscle belly.

**Fig. 3.1.24**

(A) Ventral medial compartment of the right guttural pouch. (B) Ventral medial compartment of the right guttural pouch with figure labels. ICA, internal carotid artery; IX, glossopharyngeal nerve; XII, hypoglossal nerve; ECA, external carotid artery; Pharyngeal br. X, pharyngeal branch of the vagus nerve; Pharyngeal br. IX, pharyngeal branch of the glossopharyngeal nerve.

**Fig. 3.1.25**

(A) Lateral compartment of the right guttural pouch. (B) Lateral compartment of the right guttural pouch with figure labels.

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Lower airway function: responses to exercise and training

Dorothy M. Ainsworth

Physiologic responses to exercise

Overview

During high-intensity exercise, the metabolic demands of the horse — oxygen consumption and carbon dioxide production — increase more than 30-fold relative to resting conditions. As a consequence, the ventilatory output must increase in order to meet gas exchange requirements and to aid in heat dissipation. Despite the recruitment of respiratory muscles that produce large inspiratory and expiratory pressures, complemented by the generation of locomotory-associated intrathoracic and intra-abdominal pressures, the ventilatory response of the horse during high-intensity exercise is inadequate. This finding, along with the lack of training-induced improvements in respiratory system output, has led many physiologists to conclude that the respiratory system of the horse is the major limiting factor to athletic performance.

Structure and function of the lower respiratory tract

The intrathoracic trachea bifurcates into the right and left mainstem bronchi at the level of the fifth or sixth intercostal space and enters the hilum of each lung. At its division from the trachea, the right bronchus assumes a straighter, more horizontal position relative to the left bronchus. Each bronchus subsequently divides into lobar, segmental, and subsegmental bronchi with the eventual formation of bronchioles. In the distal part of the bronchial tree, the terminal bronchioles lead into poorly developed respiratory bronchioles or open directly into alveolar ducts.¹ The

tracheobronchial lining consists of tall columnar, pseudostratified epithelium interspersed with serous and goblet cells.² This is supplanted by short ciliated cells and non-ciliated Clara cells of the bronchioles, and then by type I and type II pneumocytes at the level of the alveoli.³ Type I pneumocytes, with thin cytoplasmic extensions 0.2–0.5 mm thick, cover the majority of the alveolar surface.³ The cuboidal type II cells, with their characteristic lamellar cytoplasmic inclusions that form surfactant, are also considered to be ‘stem cells’, replacing the type I cells in lung injury. Also scattered throughout the lower respiratory tract are lymphocytes, macrophages, mast cells, and occasional eosinophils, cell types that are critical for the development of pulmonary immune responses.⁴

The pulmonary structures are subserved by two vascular beds. The major source of blood is via the pulmonary circulation, a low-pressure, low-resistance system (during resting conditions) which participates in gas and heat exchange at the alveolar level and provides nutrients to the alveolar constituents. The distribution of the pulmonary arterial flow to the various lung regions in the resting horse does not appear simply to reflect the effects of gravitational forces or the effects of pressure gradients between the pulmonary arteries, veins, and alveoli as originally proposed.⁵ Using microspheres to examine the distribution of pulmonary blood in resting Thoroughbreds, Hlastala and colleagues (1996) found that blood flow to the cranial-most portions of the lungs was uniformly low (in three of the four horses) and that blood flow increased linearly with the vertical height of the lung (opposite to gravity).⁶ Hlastala also found a great deal of heterogeneity of blood flow within a single isogravitational plane, suggesting that pulmonary blood flow is not simply related to pressure gradients between pulmonary arteries, veins, and the alveoli. Otherwise,

at a given vertical height, the distribution of blood flow would have been homogeneous. The investigators suggested that the reduction of pulmonary blood flow to the ventral lung regions might reflect the greater length and resistance of the vessels bringing blood to these areas.

With just mild exercise (trotting), there is a redistribution of blood flow to the dorsal lung region but no improvement in the heterogeneity of blood flow at a given isogravitational plane.⁷ The increased flow to the dorsal lung regions may simply reflect regional differences in vascular reactivity,⁸ regional differences in structure (volume density of capillaries), and/or changes in pulmonary vascular pressures.⁷

The bronchial circulation, a high-pressure circulatory bed, is the second source of blood flow to the lungs. At its origin from the aorta, it is a single vessel that subsequently divides into a right and left bronchial artery at the hilar region of the lung. The artery courses along the bronchi and provides nutrients to the lymphatic, vascular, and airway components, and supplies arterial blood to the pleural surface.¹ In ponies exercising at 6 m/s (7% incline) for nearly 30 min, the bronchial blood flow was found to increase 16-fold relative to resting levels.⁹ The increase in blood flow, attributed to a decrease in vascular resistance, was highly correlated with the exercise-induced increases in the pulmonary artery temperature.

The pulmonary structures are innervated by parasympathetic, sympathetic, and non-adrenergic non-cholinergic (NANC) pathways.¹⁰ The relative contributions of these pathways to the airway tone in healthy resting and exercising horses have been examined. Neither muscarinic blockade of the parasympathetic system (the vasoconstrictor system) nor β_2 -adrenergic activation of the sympathetic system (the vasodilator system) alters resting airway diameter.

The major function of the lung is gas exchange — uptake of oxygen and elimination of carbon dioxide. During resting conditions, the horse, utilizing a breathing frequency of 12–15 breaths per minute and a tidal volume of nearly 6 liters, produces a total minute ventilation of approximately 72–80 L/min.¹¹ During this same period, the horse consumes approximately 2.1 L/min of oxygen (3000 L/day) and produces approximately 1.7 L/min of carbon dioxide (2400 L/day). Thus the lung provides an important means by which normal arterial oxygen and carbon dioxide tensions and arterial pH are maintained. As discussed below, impairment of this pulmonary function becomes evident in horses exercising at

intensities exceeding 60–65% of maximum oxygen consumption.¹²

Additional functions of the lung include metabolism of bioactive amines (pulmonary circulation), production of surfactant, and the maintenance of pulmonary defense mechanisms. The lower respiratory tract also aids in thermoregulation through evaporative heat losses. It has been estimated that approximately 25% of the heat load generated during low-intensity exercise is dissipated through the respiratory tract.¹³

Pulmonary function testing

In resting horses, respiratory mechanics (tidal and minute volume, breathing frequency, dynamic lung compliance, and total pulmonary resistance) have been routinely measured using flow meters and esophageal balloon catheters (Table 3.2.1) or using the technique of forced oscillation.¹⁸ Measurement of lung volumes other than tidal and minute volume requires the use of the inert gas dilution technique (end-expiratory lung volume, EELV) and the construction of quasistatic pressure–volume curves obtained in anesthetized or heavily sedated horses (total lung capacity (TLC), residual volume (RV)).^{19,20} Estimates of TLC range from 45 to 55 liters while those of RV range from 9 to 10 liters (Fig. 3.2.1). The variability in the lung volume measurements may be due, in part, to positional and anesthetic effects.^{19,21} End-expiratory lung volume measurements in awake horses using helium dilution or nitrogen washout techniques yield values ranging from 20 liters to 35

Table 3.2.1 Range of ventilatory parameters in horses during eupneic breathing

V_T (L)	4.9	5	4.1	5.7
fb (per min)	15.5	14.5	14.7	9.6
$\dot{V}_E$ (L/min)	75	68	60	55
C_{dyn} (L/cmH ₂ O)	2.3	1.3	—	3.2
R_L (cmH ₂ O/L/s)	—	0.36	0.61	0.43
Reference	14	15	16	17

V_T , tidal volume; fb, breathing frequency; $\dot{V}_E$, expired minute ventilation; C_{dyn} , dynamic compliance; R_L , pulmonary resistance. Reproduced from Aguilera-Tejero E, Pascoe JR, Smith BL, Woliner MJ Research in Vet Sci 1997; 62:144, by permission of WB Saunders, and Lavoie JP, Pascoe JR, Kupersmoek JJ American J Vet Res 1995; 56(7):926, by permission of American Veterinary Medical Association.

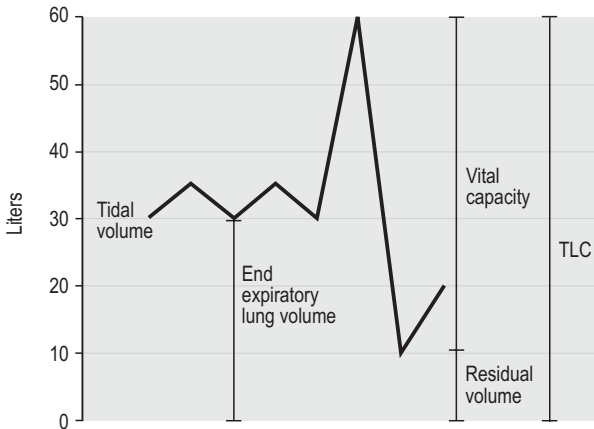


Fig. 3.2.1

Equine lung volumes. The total lung capacity (TLC) of the horse is approximately 55 liters. During eupneic breathing, the horse uses a tidal volume of 5 liters that may increase to 18 liters during strenuous exercise. End-expiratory lung volume during eupneic breathing is 30 liters but has not been measured during exercise.

liters.^{15,17,22} Note that in horses the term EELV rather than functional residual capacity (FRC) is used to describe the volume of gas remaining in the respiratory system at the end of expiration. As expiration in humans is a passive process, FRC represents the volume resulting from the outward recoil of the chest wall and inward recoil of the lungs. Thus horses, in contrast to humans, breathe below rather than from the FRC of the respiratory system.²³ Vital capacity of the horse, the amount of air that could be inhaled following a forced expiration, ranges from 35 to 45 liters (Fig. 3.2.1).

Accurately measuring exercise-associated ventilatory parameters such as V_T , f_b , C_{dyn} , and R_L in the horse has proven to be challenging for several reasons. Breathing masks increase the respiratory tract dead space and cause rebreathing of carbon dioxide.²⁴ The breathing mask may also alter the horse's respiratory pattern such that locomotory:respiratory coupling during high-intensity exercise does not occur.^{25,26} Flow meters that are attached to the breathing mask — either pneumotachs (Fig. 3.2.2A, B) or ultrasonic flow devices (Fig. 3.2.3) — also may be problematic.²⁷ Pneumotachs offer a resistance to breathing and, when they become coated with airway secretions, overestimate actual airflow.²⁴ Open flow systems,²⁸ traditionally used to measure metabolic rates during exercise (Fig. 3.2.4), have been intermittently converted to closed ventilatory systems, using pneumotach-like devices. The respiratory parameters of 5–10

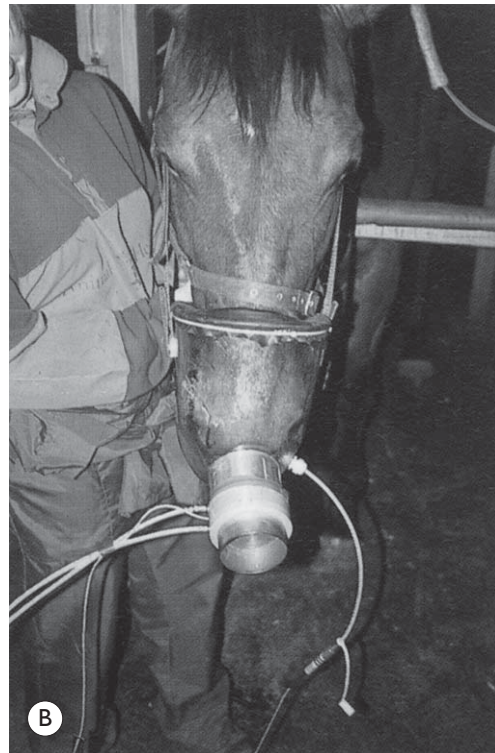
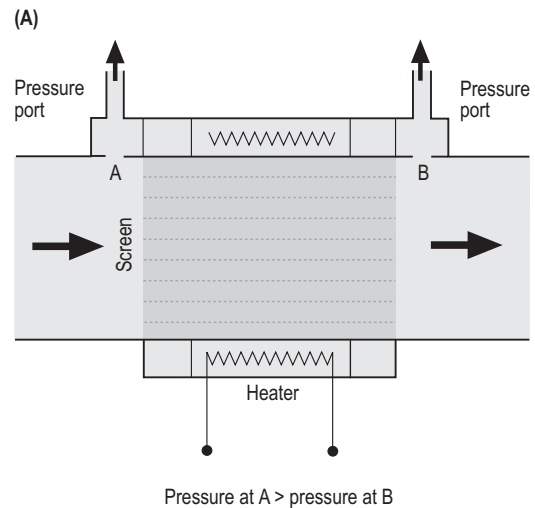
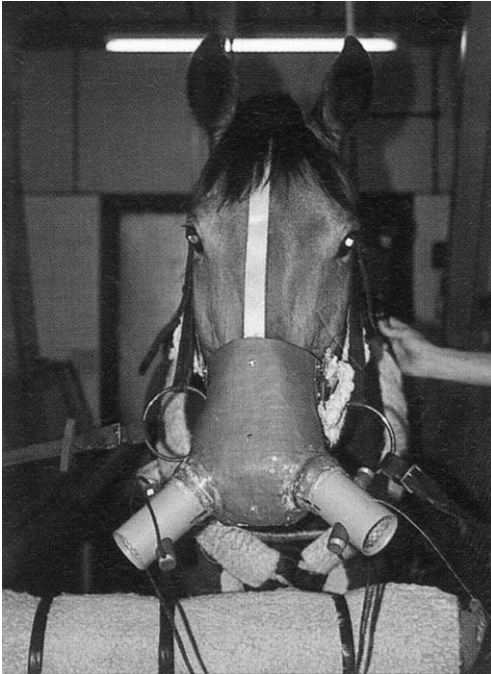


Fig. 3.2.2

Flow meter. (A) Diagram and (B) photograph of a pneumotach attached to a breathing mask. This device is used to measure airflow in resting and exercising horses. The pressure difference across a heated screen is detected during breathing and transduced to a flow signal. Pneumotachs may become coated with airway secretions. (Reproduced with permission from Marlin & Roberts.²⁷)

**Fig. 3.2.3**

Ultrasonic flow meter. Photograph of a horse wearing a breathing mask with two ultrasonic flow meters centered over each nostril. This may offer less resistance to breathing than the pneumotach, but may suffer from baseline drift. (Reproduced with permission from Marlin & Roberts.²⁷)

breaths are obtained before gas exchange impairment occurs (rebreathing CO_2) or before respiratory secretions accumulate on the pneumotachograph.²⁴ Ultrasonic flow detectors have also been used in place of pneumotachs. These devices may exhibit baseline drift due to moisture buildup and, because they are sensitive to gas densities, are unable to be used in studies examining the effects of various gases (heliox) on respiratory mechanics.²⁷

Because of technical difficulties, exercise-associated changes in lung volumes (TLC, EELV) have not been measured in the horse, confounding the interpretation of flow:volume loops (see 'Mechanical factors limiting ventilation', below). It is not known if TLC remains unchanged in the horse as it does in the human, as the determinants of TLC — respiratory muscle strength, lung and chest wall recoil — have not been measured in exercising horses.²⁹ In humans, FRC decreases with exercise but when high workloads are imposed, EELV may then increase towards or above normal due to expiratory flow limitations.³⁰

Although EELV has not been measured in the horse, exercise-induced increases in the end-expiratory costal diaphragmatic length (i.e. lengthening of the diaphragm prior to initiation of inspiration), detected by sonomicrometry techniques, suggest that EELV decreases.³¹ The actual volume change in EELV is unknown.

Response of the respiratory system to exercise

Metabolic demand

The exceedingly high metabolic demands of strenuous exercise in the horse must be met by corresponding increases in the output of the respiratory and cardiovascular systems. The magnitude of exercise-induced changes in mean oxygen consumption ($\dot{V}\text{O}_2$), carbon dioxide production ($\dot{V}\text{CO}_2$) and in heart rate (HR), as a function of running speed, are depicted in Figure 3.2.5. Note that $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ increase linearly up to speeds of 10 m/s and thereafter change little, as evidenced by the plateau.³² Exercise speeds required to produce 115% $\dot{V}\text{O}_{2\text{ max}}$ would thus be extrapolated by extending the linear portion of the exercise speed- $\dot{V}\text{O}_2$ relationship. The average maximum oxygen consumption in horses performing incremental exercise tests has been reported to be 138 mL/kg/min in Standardbreds³³ and 142 mL/kg/min in Thoroughbreds.³⁴ Values as high as 190 mL/kg/min for individual horses have been recorded. Relative to the resting value of 4–5 mL/kg/min, this represents a 30-fold increase in oxygen consumption during strenuous exercise!

Exercise-associated increases in $\dot{V}\text{O}_2$ are mediated by increases in both oxygen delivery and tissue extraction. Delivery of oxygen is enhanced not only by increases in cardiac output, but also by increases in hemoglobin (red cell numbers) that result from splenic contraction. The spleen, which stores approximately one-third to one-half of the horse's total red blood cells, is the source of the 1.7-fold increase in hemoglobin at the onset of exercise.³⁵ Indeed, splenectomy³⁶ reduces maximum oxygen consumption by approximately 31%. As each gram of hemoglobin is capable of binding 1.3 mL of oxygen, the total arterial oxygen content (CaO_2), the sum of dissolved and bound oxygen, increases from a resting value of approximately 21 mL/dL to a value of 27 mL/dL in strenuously exercising horses (Table 3.2.2).³⁷

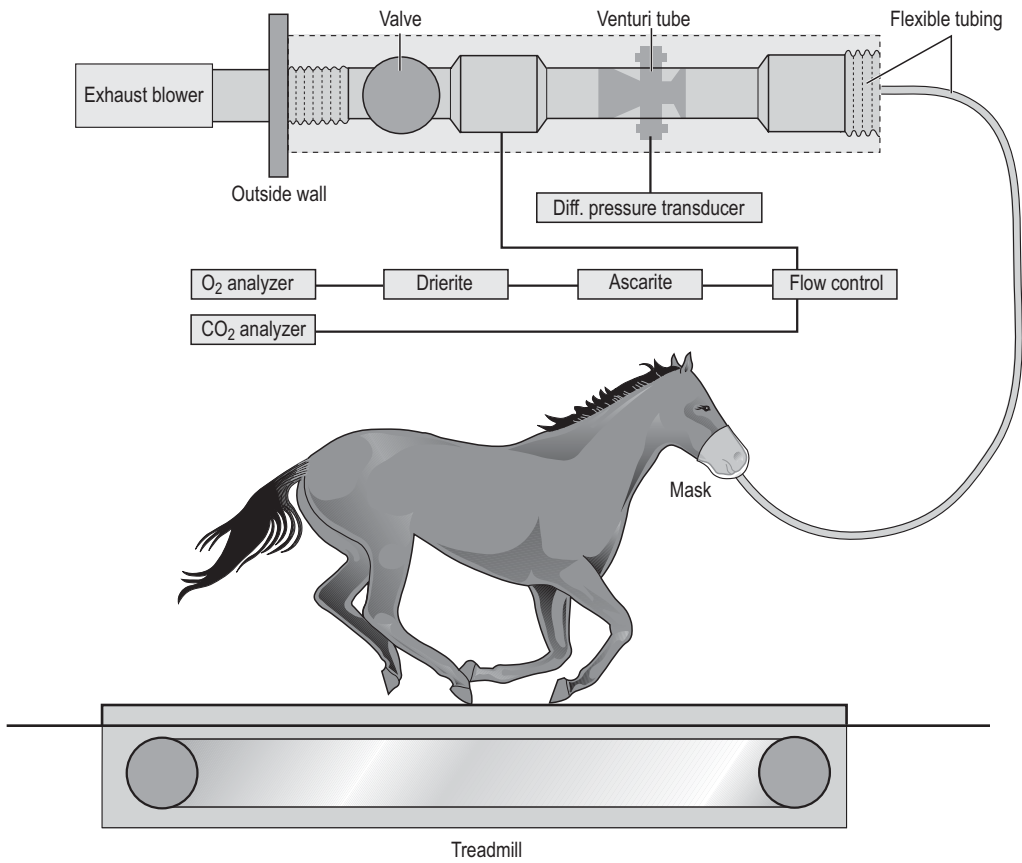
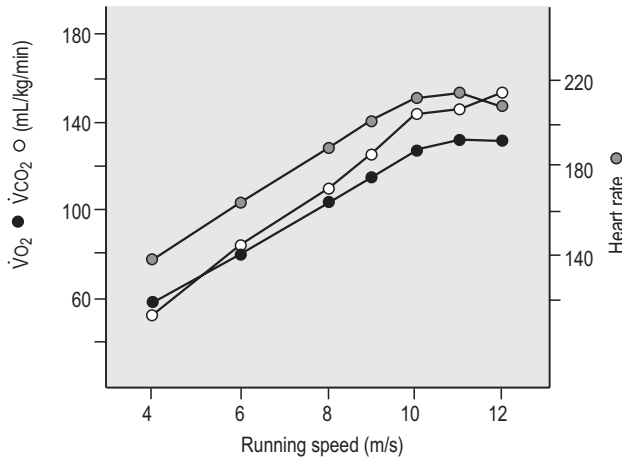
**Fig. 3.2.4**

Diagram of an open flow system used to measure expired carbon dioxide and consumed oxygen during exercise. (Reproduced with permission from Seeherman & Morris.²⁸)

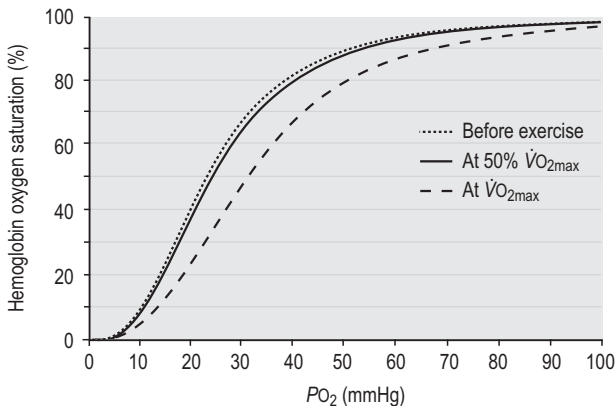
Table 3.2.2 Blood gas data from Thoroughbreds performing an incremental exercise test on a treadmill

	Rest	6 m/s	8 m/s	14 m/s 3.5° incline	Recovery 5-min walk
pH _a (mmHg)	7.42	7.44	7.41	7.21	7.13
PaCO ₂ (mmHg)	43.9	36.5	39.6	50.0	23.8
PaO ₂ (mmHg)	101.2	104.2	100.9	73.5	96.9
CaO ₂ (mL/dL)	20.6	26.4	27.1	26.5	27.5
SaO ₂ (%)	99.0	99.0	98.2	89.3	96.9
PvO ₂ (mmHg)	40.3	27.7	23.1	14.0	49.6
CvO ₂ (mL/dL)	16.4	14.3	9.4	2.3	17.4

pH_a, arterial pH; PaCO₂, arterial carbon dioxide tensions; PaO₂, arterial oxygen tensions; CaO₂, arterial oxygen content; SaO₂, arterial oxygen saturation; PvO₂, mixed venous oxygen tensions; CvO₂, mixed venous oxygen content. Reproduced with permission, from Manohar et al.³⁷

**Fig. 3.2.5**

Metabolic demands of exercise. The mean exercise-associated increases in carbon dioxide production ($\dot{V}CO_2$), oxygen consumption ($\dot{V}O_2$), and heart rate (HR) in Thoroughbred horses performing an incremental exercise test. (Reproduced with permission from Rose et al.³²)

**Fig. 3.2.6**

Oxyhemoglobin dissociation curve. With exercise, there is an increase in tissue temperature, carbon dioxide production, and hydrogen ion concentration, which facilitates unloading of oxygen (right shift of oxyhemoglobin dissociation curve) at the tissue capillary. (Reproduced with permission from Fenger et al.³⁸)

Extraction of oxygen at the tissue level is enhanced by a right shift of the oxyhemoglobin dissociation curve (Fig. 3.2.6), responding to increases in carbon dioxide tensions, hydrogen ion concentrations, or elevations in tissue temperatures. The net result is that the amount of dissolved oxygen (PO_2) is increased as oxygen is 'unloaded' from the hemoglobin. This

improves the oxygen gradient between the tissue capillary and the cell mitochondria, enhancing diffusion into the cell. In the horse, the primary mediator of the Bohr effect, the rightward shift of the oxyhemoglobin dissociation curve, appears to be the metabolic acidosis of exercise.³⁸

The magnitude of tissue oxygen extraction during exercise, the difference between the arterial (CaO_2) and venous oxygen content (CvO_2), can be appreciated from the data presented in Table 3.2.2. At rest, the horse utilizes approximately 4 mL/dL of oxygen as opposed to the nearly 25 mL/dL consumed when the horse gallops at 14.5 m/s on a grade. Understandably, venous oxygen tensions are reduced from 40 torr at rest to 12 torr during maximal exercise. Although, as anticipated, ventilation increases during exercise, the response is insufficient to prevent the development of arterial hypoxemia ($PaO_2 < 85$ mmHg) and hypercapnia ($Paco_2 > 45$ mmHg) (see 'Gas exchange during exercise', below).

Ventilatory output

During exercise, the magnitude of the increase in ventilatory output will be a function of the intensity and of the duration of exercise. Representative changes in ventilatory parameters are shown in Table 3.2.3 for Thoroughbreds¹¹ and in Table 3.2.4 for Standardbreds³⁹ performing an incremental exercise test.

As shown in Table 3.2.3, tidal volume (V_T) and minute ventilation ($\dot{V}_E$) increase linearly with treadmill speed and correlate with increases in inspiratory muscle activity.⁴⁰ Breathing frequency (fb) increases linearly with speed from rest to 8 m/s but, at faster speeds, only slight increases in breathing frequency occur. In Thoroughbreds, breathing frequency is entrained or linked to stride frequency during the canter and gallop, a phenomenon termed locomotory: respiratory coupling (LRC).⁴¹ Breathing frequency may or may not be coupled to stride frequency during trotting and is usually not linked to stride frequency during walking.⁴² Interestingly, despite the development of LRC, stride length and V_T are not tightly coupled during the canter or gallop. This enables V_T to be increased independently in response to metabolic need or demand.⁴³ That is, in horses galloping at a constant speed on a treadmill, increases in the treadmill incline (and in $\dot{V}O_2$) induce increases in minute ventilation and in V_T without altering stride length.

In contrast to the exercising Thoroughbred, the trotting Standardbred utilizes a slightly different

Table 3.2.3 Ventilatory parameters of Thoroughbred horses during an incremental exercise test and during recovery

	Rest	Walk 1.6 m/s	Trot 3.4 m/s	Canter 8 m/s	Gallop 10 m/s	Gallop 12 m/s	Gallop 10 m/s, 2°	Gallop 10 m/s, 4°	Recovery 10-min walk
V_T (L)	4.8	5.8	6.4	9.2	11.5	12.4	12.3	13.2	6.5
fb (per min)	16	65	91	113	122	126	118	121	126
$\dot{V}_E$ (L/min)	77	361	564	1042	1335	1562	1453	1585	777
Peak $\dot{V}_E$ (L/s)	5.1	14.3	27.6	45.3	55	65.2	60.2	63.9	39.2
Peak $\dot{V}_I$ (L/s)	5.1	17.9	30.5	52.5	66.6	77.9	68.5	78.8	31.6
$\Delta P_{pl\ max}$ (cmH ₂ O)	4.4	15.6	24.2	56.1	73.4	84.2	77.5	83.9	22.0
Wrm (J/L)	0.41	1.20	2.41	3.82	5.16	6.07	5.37	6.22	2.1
R_L (cmH ₂ O s/L)	0.20	0.25	0.30	0.49	0.53	0.52	0.53	0.55	0.25
$\dot{V}_{O_2}$ (mL/kg/min)	4.6	20.2	25.3	85.4	114.3	124.2	124.4	139.3	27.7
$\dot{V}_{CO_2}$ (mL/kg/min)	3.8	15.9	23.1	80.6	113.5	136.2	123.9	142	29.8
PaO_2 (mmHg)	92	102	99	83	77	69	72	70	116
$PaCO_2$ (mmHg)	47	46	43	46	49	53	49	49	33

V_T , tidal volume; fb, breathing frequency; $\dot{V}_E$, expired minute ventilation; peak $\dot{V}_E$, peak expiratory flow; peak $\dot{V}_I$, peak inspiratory flow; $\Delta P_{pl\ max}$, difference between peak inspiratory and peak expiratory pressure; Wrm, work of breathing; R_L , pulmonary resistance; $\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{CO_2}$, carbon dioxide production; PaO_2 , arterial oxygen tensions; $PaCO_2$, arterial carbon dioxide tensions.

Note during the last two exercise levels, the treadmill was inclined 2 and 4° respectively.

Reproduced with permission, from Art et al.¹¹

Table 3.2.4 Ventilatory parameters of Standardbred horses during an incremental exercise test

	Rest	Walk 1.7 m/s	Trot 4 m/s	Trot 7 m/s	Trot 8 m/s	Trot 9 m/s	Trot 10 m/s
V_T (L)	5	5	9	13	15	17	20
fb (per min)	19	79	79	79	90	90	90
$\dot{V}_E$ (L/min)	95	395	711	1027	1350	1530	1800
pHa (mmHg)	7.37	7.42	7.41	7.37	7.32	7.24	7.22
$PaCO_2$ (mmHg)	44	45	43	46	47	48	51
PaO_2 (mmHg)	99	110	99	88	84	80	75
BE (mmol/L)	1.2	1.9	1.9	0.9	-1.4	-4.1	-7.1
$\dot{V}_{O_2}$ (mL/kg/min)	11	29	55	104	133	157	166
$\dot{V}_{CO_2}$ (mL/kg/min)	9	23	53	95	134	168	185

V_T , tidal volume; fb, breathing frequency; $\dot{V}_E$ (L/min), expired minute ventilation; pHa, arterial pH; $PaCO_2$, arterial carbon dioxide tensions; PaO_2 , arterial oxygen tensions; BE, base excess; $\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{CO_2}$, carbon dioxide production.

After an 8-minute warm-up period horses exercised for 1 minute at each of the speeds.

Reproduced with permission from Art & Lekeux.³⁹

breathing pattern.³⁹ At submaximal exercise, trotters entrain breathing frequency with stride frequency 1 : 1 but this ratio changes to 1 : 1.5, 1 : 2, or 1 : 3 at maximum exercise.⁴⁴ This increases inspiratory and expiratory times and generates a greater tidal volume. At comparable metabolic workloads ($\dot{V}_{O_2}$ of Thor-

oughbreds = 140 mL/kg/min, $\dot{V}_{O_2}$ of Standardbreds = 133 mL/kg/min), trotters exhibit a slower breathing frequency and a slightly greater V_T than Thoroughbreds (Tables 3.2.3, 3.2.4). At comparable speeds (10 m/s, 0° incline), the ventilatory output of the Standardbreds exceeds that of the Thoroughbred

horses but the greater response is commensurate with the increased metabolic workload (166 mL/kg/min versus 114 mL/kg/min).

Exercise-associated alterations in alveolar ventilation ($\dot{V}_A$) have also been measured in horses. In a study of Thoroughbreds performing an incremental exercise test, Butler and colleagues reported a 20-fold increase in $\dot{V}_A$ from the resting value (38 L/min) when horses galloped 12 m/s for 2 minutes.⁴³ The physiologic dead space to tidal volume ratio (V_D/V_T) initially increased from the resting value of 0.41 when horses trotted, but then returned to the resting value as exercise intensity increased. Although V_T , $\dot{V}_A$ and V_D increase proportionately with exercise, the increase in alveolar ventilation, relative to the increase in $\dot{V}_{CO_2}$, is insufficient to prevent hypercapnia from developing (Tables 3.2.2–3.2.4) (see ‘Gas exchange during exercise’, below).

During exercise, total pulmonary resistance (R_L) and the work of breathing increase exponentially with ventilatory output (Table 3.2.3). The exercise-associated increase in R_L is attributed to the generation of turbulent flow in the upper respiratory tract during inspiration and the narrowing of intrapulmonary airways during expiration.^{11,45,46} Understandably, pre-treatment of healthy exercising horses with bronchodilators — clenbuterol, albuterol, or ipratropium — fails to reduce total pulmonary resistance or the work of breathing.^{46,47} Note also in Table 3.2.3 that the work of breathing increases 15-fold from the resting value when the horse gallops at 12 m/s. Minimizing the work of breathing during high-intensity exercise has been suggested as a contributing cause to gas exchange failure in the athletic horse.⁴⁸

Control of breathing during rest and during exercise

Rhythmic breathing during eupnea has been attributed to the workings of a central pattern generator that, through its effects on the intermediary bulbospinal neurons of the medulla, ultimately activates inspiratory and expiratory motoneuron pools of the spinal cord. In the resting horse both inspiration and expiration are active processes reflecting the electromechanical activation of the diaphragm (inspiratory muscle) and of the transverse abdominal and external oblique muscles (expiratory muscles).^{22,23} The role of the rib cage muscles in generating the breath has not been well studied in the horse.

During eupneic breathing, the initial generation of inspiratory flow precedes electrical activation of the diaphragm and is attributed to outward recoil of the chest wall and relaxation of abdominal expiratory muscles (Fig. 3.2.7). With diaphragmatic activation, inspiratory airflow again increases, causing the biphasic flow pattern. During expiration, relaxation of the diaphragm contributes to the initial generation of expiratory flow. Once expiratory muscles are activated, there is a further increase in expiratory flow.

When horses are exercised, linear increases in the diaphragmatic electromyogram (EMG) are associated with linear increases in the transdiaphragmatic pressure (Fig. 3.2.8).⁴⁰ Abdominal muscles are also recruited during the exercise hyperpnea. In ponies walking on the treadmill, there is a temporal correlation between the development of peak transverse abdominal EMGs and peak positive esophageal pressure, suggesting an expiratory function for this muscle.⁴⁹ In contrast, the EMGs of the rectus abdominis and abdominal oblique muscles in horses during mild to high-intensity exercise exhibit a locomotory-associated modulation.⁵⁰

The generation of a breath during eupnea or during the exercise hyperpnea is shaped by inputs from:

- central and peripheral chemoreceptors
- mechanoreceptors of the intra- and extrathoracic airways and lung parenchyma
- phrenic afferents
- locomotory-associated stimuli
- higher central nervous system (CNS) centers.

Chemoreceptors

Chemoreceptors are sensors that detect changes in CO_2 , O_2 , and pH, and have been classified, based upon anatomical location, as either central or peripheral. At a given pH, the ventilatory response of the central chemoreceptors, presumed to be located in the medulla, is greater during a respiratory acidosis than during a metabolic acidosis. The augmented response is attributed to the more soluble CO_2 that easily permeates the blood–brain barrier, activating the central chemoreceptors. In awake chronically instrumented horses, activation of central chemoreceptors by hypercapnic challenge augments both inspiratory and expiratory muscle activation (Fig. 3.2.7), leading predominantly to a V_T response with little change in breathing frequency.²²

Peripheral chemoreceptors are located at the bifurcation of the carotid arteries and predominantly detect

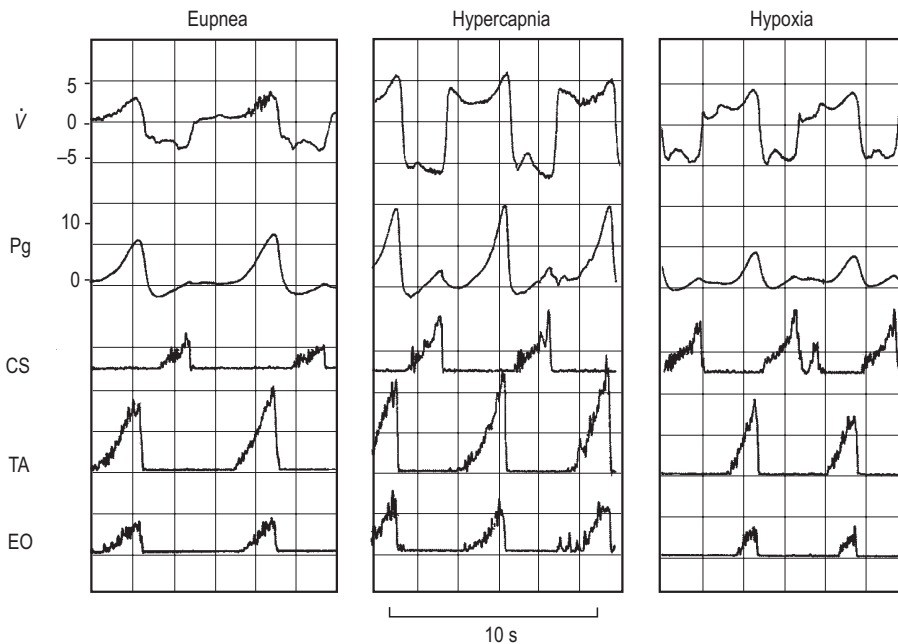


Fig. 3.2.7

Relationship between respiratory flow ($\dot{V}$), gastric pressure changes (P_g), and respiratory muscle (costal diaphragm (CS), transverse abdominal (TA), and external abdominal oblique (EO)) Electromyograms during eupneic, hypercapnic, and hypoxic breathing. At rest, initial inspiratory flow precedes activation of the diaphragm and is attributed to relaxation of expiratory muscles (decrease in P_g). Similarly, the initial generation of expiratory flow precedes abdominal expiratory muscle activation and is attributed to relaxation of inspiratory muscles. With hypercapnic challenge, inspiratory and expiratory muscles are recruited to increase tidal volume and minute ventilation. Hypoxia causes an increase in inspiratory muscles but less of an increase in abdominal expiratory muscles. (Reproduced with permission from Ainsworth et al.²²)

changes in oxygen tensions. Activation of peripheral chemoreceptors via hypoxic challenge increases the magnitude and frequency of inspiratory muscle activation with little change in abdominal expiratory muscle activity (Fig. 3.2.7).²²

Although arterial hypoxia and hypercapnia develop during exercise,^{12,51} it does not appear to be the result of impaired chemoreception. When horses breathe a hyperoxic mixture (inspired fraction ($F_{I\text{O}_2}$) = 0.3) while galloping at 14 m/s, there is a reduction in $\dot{V}_A$ relative to normoxic trials — an expected response. When the $F_{I\text{O}_2}$ is reduced from 0.21 to 0.16 in those horses galloping at 14 m/s (causing a corresponding decrease in $P_{a\text{O}_2}$ from 56 to 38 torr), tidal volume and minute ventilation increase 20%, confirming that an intact hypoxic drive exists.⁴⁸ Interestingly, when exercising horses (14 m/s) breathe a hypercapnic gas mixture ($F_{I\text{CO}_2}$ = 0.06) causing $P_{a\text{CO}_2}$ to increase from 50 to 80 torr, ventilation fails to increase in the majority of horses studied.⁵² The occasional horse will

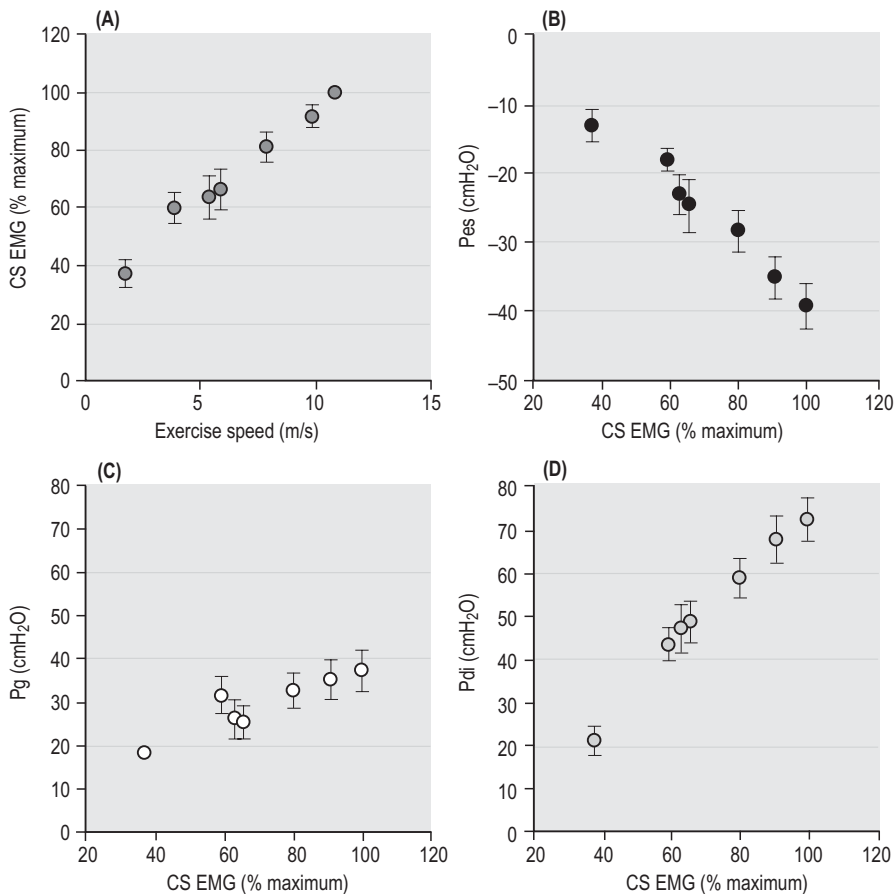
uncouple breathing with limb movement to increase tidal volume.

The question of why the horse does not increase ventilation in response to the decrease in $P_{a\text{O}_2}$ (65% $\dot{V}\text{O}_{2\text{ max}}$) — when mechanical flow limitations do not exist — is unknown. Some investigators have suggested that this breathing strategy simply reflects that of a 'smart' ventilatory controller that 'chooses' to minimize the mechanical cost of breathing rather than to optimize blood gas tension and acid-base balance.⁴⁸

The question of why the horse develops hypercapnia at near-maximal exercise is discussed below in the section on 'Mechanical factors limiting ventilation'.

Mechanoreceptors

Breathing pattern is also influenced by mechanoreceptive input from receptors within the airways of the respiratory system, within the costovertebral

**Fig. 3.2.8**

Relationship between diaphragmatic activation and mechanical output during incremental exercise in horses. In panel A, the linear increase in the diaphragm (CS) EMG that occurs as horses exercise at faster treadmill speeds is demonstrated. The increase in electrical activity of this inspiratory muscle is associated with a progressive decrease in peak esophageal pressure (Pes, panel B), a progressive increase in peak inspiratory gastric pressure (Pg) as the diaphragm descends into the abdominal cavity during inspiration (panel C) and a linear increase in the mechanical output of the diaphragm, the transdiaphragmatic pressure (Pdi), during exercise. (Reproduced with permission from Ainsworth et al.⁵⁰)

articulations and within the rib cage and abdominal musculature (spindles, Golgi tendon organs). Within the lung, three types of pulmonary mechanoreceptor have been identified: the slowly adapting receptors (SARs), the rapidly adapting receptors (RARs), and the non-myelinated C fibers.⁵³ Vagally mediated inputs from the SARs, responding to increases in lung inflation, feed back on to the central respiratory controller to terminate inspiration and to activate expiratory muscles. The RARs are mechanoreceptors with a primary function of mediating augmented breaths or sighs. Changes in lung compliance during eupneic breathing are thought to be sensed by RARs, which then initiate sighs. Pulmonary and bronchial C fibers, vagally mediated non-myelinated fibers, are activated

by substances produced, released, and catabolized in the lungs (bronchial C fibers) or by mechanical alterations in the lung parenchyma that occur with congestion and edema (pulmonary C fibers). Their contribution to the control of breathing in the horse has not been investigated, but in other species, activation results in a tachypneic pattern.⁵³

In resting ponies, elimination of mechanoreceptor input, either by vagal cooling or by local anesthesia, prolongs inspiratory time, decreases breathing frequency and R_L , and increases V_T , but has no effect on arterial blood gas tension, minute ventilation, and dynamic compliance.⁵⁴ In ponies performing mild treadmill exercise (walking, trotting), removal of vagally mediated afferent inputs via hilar denervation

produces similar effects. There is an increase in V_T , a decrease in breathing frequency, and a preservation of minute ventilation and arterial blood gases.⁵⁵ Thus, during low-intensity exercise, mechanoreceptor inputs are not critical for the exercise hyperpnea to develop. However, the effects of vagal deafferentation on the pattern of breathing (locomotory:respiratory coupling) or on gas exchange in horses performing high-intensity exercise remain to be determined.

Phrenic afferents

The diaphragm and the non-respiratory muscles are innervated by small afferents (types III and IV) that respond to mechanical and chemical stimuli.⁵⁶ Although the majority of studies examining the effects of phrenic afferents on the control of breathing have been obtained in studies of anesthetized cats and dogs, the data confirm muscle afferents to be powerful stimuli to ventilation. In lightly anesthetized dogs, electrical stimulation of phrenic afferents causes a 500% increase in ventilation — a response equivalent to that induced with breathing 10% CO_2 .⁵⁷ Nevertheless, it does not appear that diaphragmatic afferents are the primary drive for the exercise hyperpnea, as diaphragmatic deafferentation does not affect ventilation or arterial carbon dioxide tensions in ponies that are mildly exercised.^{58,59}

Locomotory-associated stimuli

Thoroughbred horses routinely entrain or couple breathing frequency with limb movement.^{41,42} The mechanism of this coupling has not been established but it has been postulated to involve spinal afferents. Evidence for this comes from a study in ponies with partial spinal cord ablation. The net effect of the intervention is to attenuate the exercise-induced increases in breathing frequency, suggesting that feedback from limb movement modifies the exercise hyperpnea.⁶⁰

In addition to neural inputs that would affect the pattern of breathing during exercise, locomotory-associated forces have also been suggested to contribute significantly to the exercise hyperpnea. A number of benefits could possibly derive from integration of locomotion and respiration since locomotion might affect the mechanical characteristics of the respiratory system by stiffening the chest wall, by reducing respiratory system compliance, and by increasing the work of breathing.

Three exercise-associated forces postulated to generate airflow in horses during locomotion include:

- the to and fro movements of the liver and intestines (visceral piston) effecting diaphragmatic movement
- the concussive forces resulting from limb impact that are transmitted to the thoracic cavity to produce pressure and volume changes
- the compressive forces developing within the abdominal cavity during lumbosacral flexion and extension that ultimately produce pressure and volume changes within the thoracic cavity.

Although this biomechanical model of ventilation fits well with the observed locomotory movements and respiratory airflow patterns in galloping horses, little conclusive evidence exists to support their relative contributions to the exercise hyperpnea. Young and colleagues have estimated that the visceral displacements are 230° out of phase with ventilation.⁶¹ They suggested that lumbosacral flexion and extension exerted a more significant biomechanical effect on ventilation. Frevert and colleagues also studied the breathing pattern of galloping horses that occasionally departed from the 1:1 LRC ratio.⁶² By ensemble averaging the horse's respiratory flow signals using limb frequency as a trigger, they were able to calculate the contribution of limb concussive forces to ventilation. They found that stride-related volume excursions averaged 10–20% of the tidal volume. Finally, EMG recordings of respiratory muscles obtained in chronically instrumented exercising horses have clearly demonstrated that increases in phasic electrical activity of the diaphragm correlate with increases in transdiaphragmatic pressure generation independent of LRC (Fig. 3.2.8).⁴⁰

CNS inputs

Behavioral and thermal inputs from higher CNS centers influence the pattern of breathing during eupnea and may also exert modifying influences on ventilation during prolonged exercise in the horse.^{13,63} For example, when Thoroughbreds exercise at 40% $\dot{V}_{O_{2\max}}$ for 60 minutes, arterial carbon dioxide tensions decrease 10 torr further from 'steady-state' levels occurring 10 minutes into exercise. During this time, the pulmonary artery temperatures increase 2.6°C and the work of breathing nearly doubles, suggesting to the investigators that the stimulus for the ventilatory increase is a thermoregulatory one.⁶³

Other CNS inputs, specifically those radiating from locomotory-associated areas in the CNS, have also been suggested to exert a major role in the

development of the exercise hyperpnea. This idea, called the central command concept, was first proposed by Johansson and later refined in 1913 by Krogh & Lindhard.⁶⁴ Increases in ventilation during exercise are hypothesized to arise secondary to neural impulses emanating from suprapontine structures which 'command' muscles to exercise. These impulses radiate to respiratory and cardiovascular centers and thus stimulate neuronal activity, driving ventilation and respiratory muscles concurrently. The hypothesis was based primarily on the rapidity of the ventilatory and circulatory responses, which could not be accounted for by humoral mechanisms.⁶⁵ Support for the central command theory comes from studies of decorticate cats that walk on a treadmill spontaneously or during electrical or chemical stimulation of the hypothalamic locomotor regions.^{66,67} In these studies:

- the respiratory and cardiovascular responses preceded spontaneous locomotion — suggesting that the 'hyperpnea' was not dependent upon afferent feedback
- the ventilatory response was proportional to the locomotory response.

While appealing, the data have two major limitations. The decorticate cat might not duplicate physiological exercise and the metabolic rate was only minimally increased during the locomotion.⁶⁵

Gas exchange during exercise

When Thoroughbreds or Standardbreds exercise at intensities exceeding 65% of the maximum oxygen consumption ($\dot{V}O_{2\max}$), arterial hypoxemia occurs and the alveolar–arterial oxygen difference widens.^{12,44} As exercise intensity exceeds 85% of $\dot{V}O_{2\max}$, arterial hypercapnia ensues.^{12,44,51,68} This is evident in the

data presented in Tables 3.2.2–3.2.4. Gas exchange failure occurs in horses performing treadmill incremental tests and treadmill sprint tests⁶⁹ as well as in horses exercising on a racetrack.^{12,68,70}

In contrast to the horse, strenuously exercised ponies do not develop hypoxemia and hypercapnia, but rather develop an 'appropriate' ventilatory response characterized by normoxemia and hypocapnia⁷¹ (Table 3.2.5). The ventilatory equivalent — the volume of expired (or inspired) gas per volume of oxygen consumed — is 1.6-fold greater for the pony as compared to a Thoroughbred! Why the horse fails to develop an appropriate ventilatory response at submaximal exercise (i.e. hypoxemia) is unknown and does not appear to be due to a failure of chemoreception. The inadequate ventilatory response may simply reflect a breathing strategy that minimizes the exponential increase in work of breathing during exercise.⁴⁸

The mechanisms causing the arterial hypoxemia have been extensively investigated in exercising horses using a variety of approaches such as increasing the $F_{I}O_2$ or replacing inspired nitrogen with helium.^{72,73} It was not until the multiple inert gas elimination technique was adapted for use in the exercising horse that the mechanisms causing the reduced PaO_2 and the widened alveolar–arterial oxygen difference ($A-aDO_2$) could be partitioned out.^{44,74} In this technique, the airway elimination of inert gases that are dissolved in saline and infused into the venous blood is measured. The rate of elimination is dependent upon the ventilation: perfusion ratio and upon the solubility of that inert gas in the blood.⁷⁵

Hypoventilation

Although the increase in arterial (and alveolar) carbon dioxide tensions would contribute to the development of hypoxemia during exercise by reducing alveolar

Table 3.2.5 Comparison of ventilatory responses of ponies and horses at comparable metabolic work rates after 2 min of exercise

Exercise intensity	Group	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pHa	HCO_3^- (mmol/L)	$\dot{V}_E/\dot{V}O_2$
60% $\dot{V}O_{2\max}$	Thoroughbreds	81	40	7.47	29	27.6
60% $\dot{V}O_{2\max}$	Ponies	89	32	7.44	22	43.7
115% $\dot{V}O_{2\max}$	Thoroughbreds	68	50	7.26	21	26.3
115% $\dot{V}O_{2\max}$	Ponies	95	35	7.30	17	41.9

PaO_2 , arterial oxygen tensions; $PaCO_2$, arterial carbon dioxide tensions; pHa, arterial pH; HCO_3^- , bicarbonate; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent.

Reproduced with permission from Katz, et al.⁷¹

oxygen tensions, hypoventilation is not the major cause of hypoxemia.⁵¹ In fact, it only accounts for a 6–7 torr reduction in arterial oxygen tensions at the highest exercise levels. The possible causes of the hypercapnia are discussed in the section on 'Mechanical factors limiting ventilation', below.

Shunts and ventilation : perfusion inequalities

Relative to the resting condition, exercise does not cause an increase in intrapulmonary shunts. Thus, these do not contribute to the development of arterial hypoxemia.^{44,45} There is, however, a small but significant increase in the degree of ventilation:perfusion mismatch that develops with exercise. In Standard-bred trotters working at 96% of maximum $\dot{V}_{O_2}$, ventilation:perfusion mismatch accounts for 36–41% of the observed arterial hypoxemia.^{44,45} In Thoroughbreds galloping at 80% of $\dot{V}_{O_{2\max}}$, approximately 25% of the arterial hypoxemia is attributed to ventilation:perfusion inequalities.^{74,77} The cause of the ventilation:perfusion mismatch is unknown but has been hypothesized to be due to the development of low-grade interstitial edema, pulmonary hemorrhage, regional differences in pulmonary blood flow, reduced gas mixing in the large airways, or airway obstruction.⁴⁴ Overtrained horses that exhibit red cell hypervolemia also have a worsening of exercise-associated ventilation:perfusion inequalities.⁷⁶ Typically such horses develop pulmonary arterial pressures during exercise that are significantly greater than normovolemic cohorts and have an increased incidence of exercise-induced pulmonary hemorrhage.⁷⁶

Diffusion limitation

The major cause of the exercise-induced hypoxemia in the horse is a diffusion limitation.^{12,44,72,74} During exercise, the combination of rapid pulmonary blood flows coupled with a much reduced venous oxygen content have been hypothesized to cause insufficient time to achieve complete equilibration of gas exchange across the capillary–alveolar interface. Interestingly, the mean capillary transit time has been estimated using the relationship V_c/Q_t , where V_c is the total capillary blood volume (calculated from morphometric data)⁷⁸ and Q_t is the cardiac output. Estimates of capillary transit time range from 386 to 404 milliseconds in the horse^{72,76} and exceed transit times of 0.29 seconds for the dog or 0.35 seconds for the pony.⁷⁹ However, in contrast to the horse, the dog and pony do not exhibit

diffusion limitation despite markedly shortened capillary transit times. Some investigators have suggested that the horse may exhibit a greater degree of heterogeneity in the transit time or in the diffusion:perfusion that is not accounted for by simply calculating the mean capillary transit time.⁷⁴

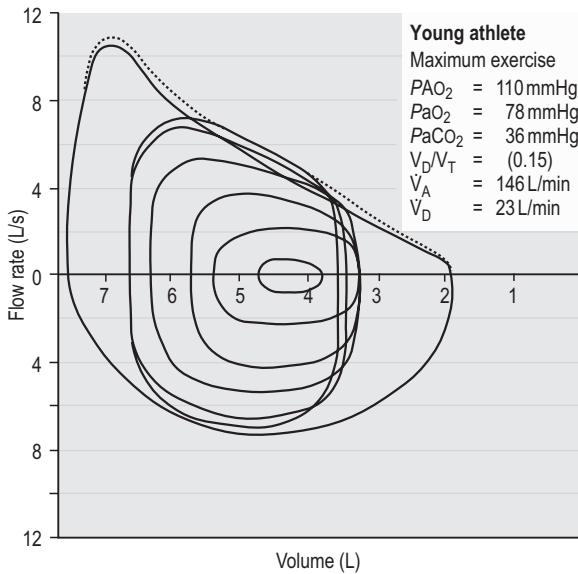
Mechanical factors limiting ventilation

The cause of the hypoventilation (hypercapnia) during high-intensity exercise is unknown but has been postulated to be due to

- an increase in dead space ventilation secondary to the high breathing frequency
- a mechanical flow limitation that results from the very short inspiratory and expiratory times; and/or
- locomotory:respiratory coupling.

If metabolic workload is held constant and the horse's breathing frequency is manipulated by changing the treadmill incline and speed, there is no effect on arterial carbon dioxide tensions.⁸⁰ This suggests that dead space ventilation is not the cause of hypercapnia. The data of Butler and colleagues also demonstrated that the ratio of dead space to tidal volume does not increase with strenuous exercise.⁴³

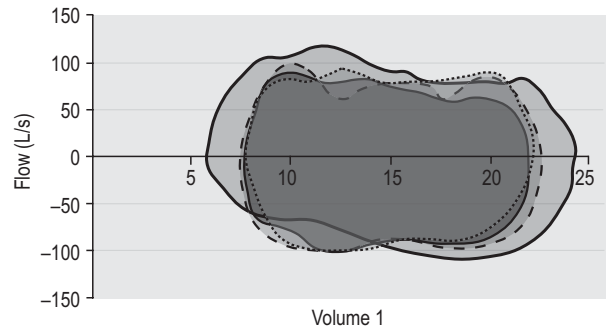
Interestingly, when fb is manipulated by altering treadmill speed and incline while preserving metabolic demand, peak expiratory flow rates change very little (85–95 L/s). This suggests the development of an expiratory flow limitation. As large expiratory pulmonary pressures are generated during exercise, dynamic compression of the non-cartilaginous airways is hypothesized to limit expiratory flow.⁸⁰ This hypothesis is supported by data obtained from horses performing strenuous exercise while breathing heliox — a gas mixture consisting of 15% oxygen and 85% helium.⁸¹ By replacing nitrogen with helium, there is a decrease in the gas density of the respired mixtures and a reduction in the turbulent flow. Thus, horses galloping at 8 m/s on a 7% incline while breathing the heliox mixture demonstrate a significant increase in $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, V_T , $\dot{V}_E$, and fb relative to the normoxic trials. Furthermore, despite an increase in metabolic workload which occurred with the heliox trials, horses were still able to increase alveolar ventilation sufficiently to reduce the severity of the arterial hypercapnia by 4 torr!

**Fig. 3.2.9**

Flow:volume loop from a human athlete performing incremental exercise. Inspiratory flow is below and expiratory flow is shown above the lung volume axis. The dashed line indicates the maximum expiratory flow obtainable in that individual. Eupneic breathing is indicated by the small loop centered near 4.25 L. With the onset of exercise, end-expiratory lung volume decreases, but as expiratory flow limitations are reached, end-expiratory lung volume increases (loop moves to the left). (Reproduced with permission from Johnson et al.⁸²)

Definitive proof of an expiratory flow limitation in exercising horses requires the measurement of flow: volume loops relative to changes in end-expiratory lung volume.⁴¹ In human athletes, expiratory flow limitations (Fig. 3.2.9) occur but may also be reduced or minimized during exercise if EELV is increased.^{30,82} However, as the athlete breathes from a higher EELV, the work of breathing is also increased. Although flow:volume loops have been measured in exercising horses (Fig. 3.2.10), it is not known whether limitation truly occurs and whether the horse can 'adjust' EELV to minimize the limitations to flow.

The question of whether LRC causes hypercapnia during high-intensity exercise was addressed in a novel study by Evans and colleagues.⁸³ They measured arterial blood gases, fb and fs (stride frequency) in Standardbred horses that were studied at comparable metabolic workloads either pacing (LRC ratio $\neq 1$) or galloping (LRC ratio = 1). At 100% $\dot{V}O_{2 \text{ max}}$, the

**Fig. 3.2.10**

Flow:volume loop from a Thoroughbred horse performing exercise at 115% $\dot{V}O_{2 \text{ max}}$. Loops were not placed relative to EELV. The four different loops were obtained while the horse exercised at 0% incline (thin solid line); 5% incline (thick dashed line); 10% incline (thin dashed line) and 20% incline (thick solid line). (Reproduced with permission from Bayly et al.⁸⁰)

$Paco_2$ of the galloping horses with strict entrainment was no different from that of the pacing horses that did not entrain breathing with ventilation. This suggested that in Standardbred horses, LRC does not impede alveolar ventilation.⁸³

In summary, expiratory flow limitation may be a plausible explanation for the development of hypercapnia during near-maximal exercise. However, one cannot discount the possibility that the pattern of breathing elected by the horse during strenuous exercise is also one chosen to minimize the work of breathing, one designed to prevent the development of diaphragmatic fatigue, and one that prevents 'steal' of blood flow from the locomotory muscles.

Physiologic responses to training

Responses and mechanisms

As training is associated with increases in aerobic power, it is logical to assume that the respiratory system would undergo training-induced adaptations to increase its ventilatory output. Evans & Rose examined the effects of a 7-week submaximal training program on respiratory responses of Thoroughbred horses.⁸⁴ Although maximal oxygen consumption increased by approximately 23% (attributed to increases in cardiac output and stroke volume),

minute ventilation remained unchanged. Evans & Rose also found a 6 torr reduction in PaO_2 tensions following training in horses exercising at 100% $\dot{V}O_{2\max}$, but this difference was not statistically significant.⁸⁴ Art & Lekeux also examined the effects of a five-step training program on cardiopulmonary and respiratory parameters in Thoroughbred horses.⁸⁵ Each step of the program lasted 3 weeks and consisted of a treadmill acclimatization period, a light exercise period (20 min of turnout), an aerobic training period (walk, trot, canter 3 days/week), an interval training period, and a detraining period. A standardized exercise test was performed after each step of the program. Although peak $\dot{V}O_2$ increased from approximately 117 mL/kg/min to 145 mL/kg/min at the end of the training program, training-induced changes in V_T , fb, and $\dot{V}_E$ were not found. The investigators did not measure concomitant changes in arterial blood gases. Christley and colleagues examined the effects of a 16-week training program on blood gases in Thoroughbreds.⁸⁶ The program consisted of an 8-week endurance phase followed by an 8-week sprint phase. They found that training significantly increased $\dot{V}O_{2\max}$ by 19%, decreased PaO_2 by 5 torr, and increased $Paco_2$ by 4 torr. The lack of a significant increase in alveolar ventilation as metabolic workload increased led to the deterioration in blood gas values. Roberts et al.⁸⁷ measured both ventilatory and blood gas parameters in Thoroughbred horses that underwent a 16-week training session that closely mimicked the one used for race horses in Great Britain. They also found a worsening of the arterial hypoxemia and hypercapnia after training (Table 3.2.6),⁸⁷ confirming the findings of previous investigators. Training does not lead to an improvement in the ventilatory parameters, and because maximum oxygen consumption and carbon

Table 3.2.6 Effects of a 16-week training program on ventilatory parameters in Thoroughbred horses galloping at 12 m/s

	Pre-training	Post-training
V_T (L)	14.6	15
fb (per min)	125	125
$\dot{V}_E$ (L/min)	1550	1800
Peak $\dot{V}_I$ (L/s)	79	80
Peak $\dot{V}_E$ (L/s)	60	60
pHa	7.25	7.30
$PaCO_2$ (mmHg)	53.6	56.5
PaO_2 (mmHg)	81	65

V_T , tidal volume; fb, breathing frequency; $\dot{V}_E$, expired minute ventilation; peak $\dot{V}_I$, peak inspiratory flow; peak $\dot{V}_E$, peak expiratory flow; pHa, arterial pH; $PaCO_2$, arterial carbon dioxide tension; PaO_2 , arterial oxygen tension.

Reproduced with permission from Roberts et al.⁸⁷

dioxide production increase, there is a worsening of the blood gases relative to the pre-training values.

In contrast to the studies that have been conducted in Thoroughbreds, there are few data evaluating the effects of training on ventilatory parameters in Standardbreds.

In summary, although training-induced modifications of the cardiac and musculoskeletal systems occur in the horse, there is a lack of pulmonary adaptations to training. This, combined with the high metabolic demands placed upon the horse during high-intensity exercise, leads one to conclude that the respiratory system is a major limitation to the athletic performance of the equine athlete.

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Cardiovascular function and oxygen transport: responses to exercise and training

David C. Poole & Howard H. Erickson

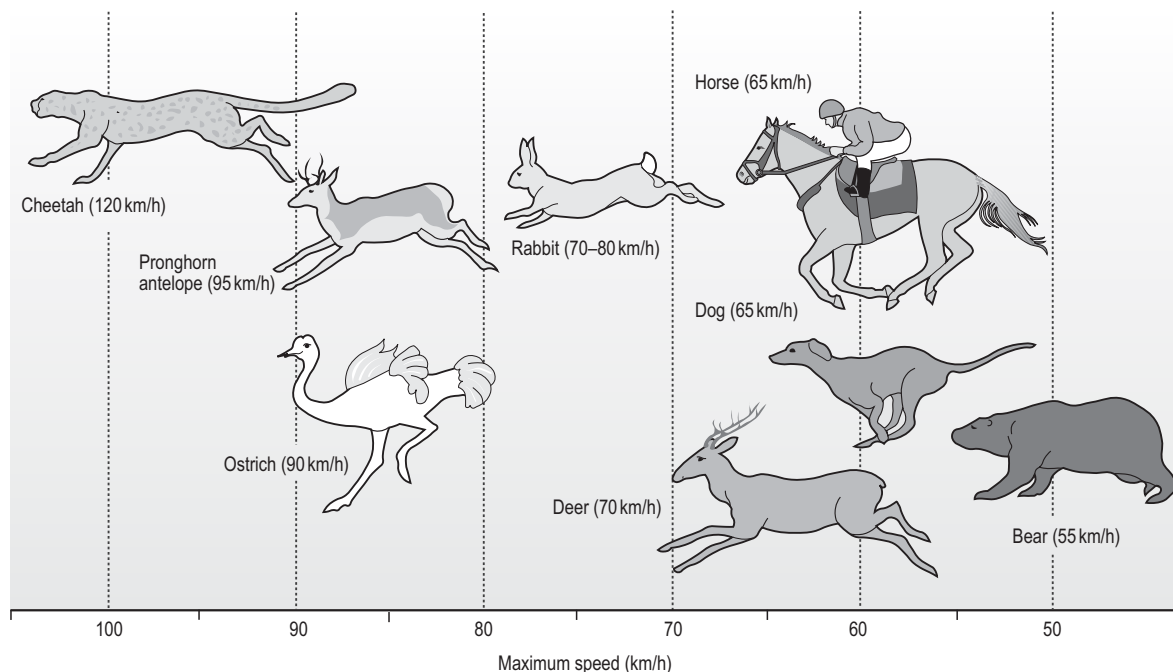
The horse is often considered one of, if not the, premier athletic mammalian species. However, depending upon the criterion used, there are other distinct (and sometimes surprising) contenders for that title. For example, as seen in Figure 4.1.1, compared with the Thoroughbred race horse (40 miles/h, 65 km/h), the cheetah can achieve speeds in excess of 70 miles/h (120 km/h) and several species of antelope, as well as the blackbuck, gnu, and ostrich, are all faster than the horse (fastest human ~27 miles/h, 43 km/h). The fastest horses ever clocked are Quarter Horses, which may reach speeds of 50–55 miles/h (90 km/h).¹ Because body length determines the distance that individual muscles shorten, it might be more appropriate to judge athletic ability in terms of speed relative to body length.² From this perspective, the Merriam kangaroo rat is superlative, achieving 110 body lengths per second, which is three times faster than the cheetah (32 lengths/s) and an order of magnitude faster than the horse (~10 lengths/s).

Within humans, the aerobic capacity or maximal oxygen uptake ($\dot{V}O_{2\text{ max}}$) is considered an excellent (though by no means the sole) indicator of performance for running events over 1 minute in duration. Across the spectrum of terrestrial mammalian species, aerobic capacity increases over five orders of magnitude as a function of body size from approximately 0.001 L/min in the 2 g Etruscan shrew (world's smallest mammal) to in excess of 80 L/min in the elite Thoroughbred race horse. Whereas it is possible that a large rapidly walking elephant may have a higher total $\dot{V}O_2$, this remains to be demonstrated. When aerobic capacity is expressed relative to body mass, the diminutive Etruscan shrew (~400 mL O_2 /kg/min)^{2,3} reigns supreme, as this measure of performance

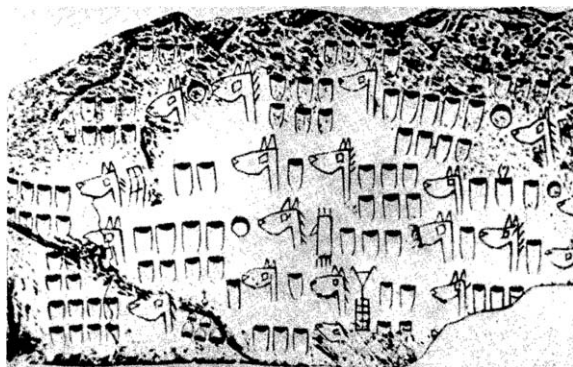
decreases systematically with increasing body mass. Amongst the larger animals, the pronghorn antelope (~300 mL O_2 /kg/min)⁴ and the horse (>200 mL O_2 /kg/min)^{2,5} are outstanding and each can consume more O_2 in toto and per unit body mass than any other mammal of their respective sizes.

This chapter addresses the structural and functional capacities of the cardiovascular system that permit the horse to achieve such prodigious O_2 flows and athletic performances. As we shall see, a large, high-capacity heart is requisite. Whereas animals such as the shrew and pronghorn antelope have evolved in accordance with the laws of nature, the horse has been subjected to several thousand years of selective breeding (Fig. 4.1.2)⁶ based upon athletic performance. As detailed below, this practice has produced a disproportionate increase in the horse's heart size and pumping capacity (cardiac output, $\dot{Q}$) compared to lung capacity; the consequence of this is a structural and functional failure of the respiratory system during maximal exercise.

The purpose of this chapter is to present those features of the equine heart and cardiovascular system that facilitate this animal's extraordinary performance. Those mechanisms underlying respiratory system failure will be discussed as they relate intimately to the cardiovascular system. Particular attention will be afforded to the plasticity of the equine cardiovascular system to exercise training and, although such changes are important, compared with the magnitude of inherited interanimal variations, they are relatively modest. Throughout this chapter and contingent upon available data, reference will be made to several other species including the human, dog, ox, and camel as a basis for comparison (Fig. 4.1.3).⁷

**Fig. 4.1.1**

Approximate maximum speeds for a variety of terrestrial mammalian species and the ostrich. Please note that the Quarter Horse has been clocked close to 90 km/h (55 miles/h)¹ whereas maximal speeds for the Thoroughbred as seen in this figure with rider are somewhat lower. For converting to other commonly used units: 10 km/h = 6.2 miles/h or 2.8 m/s.

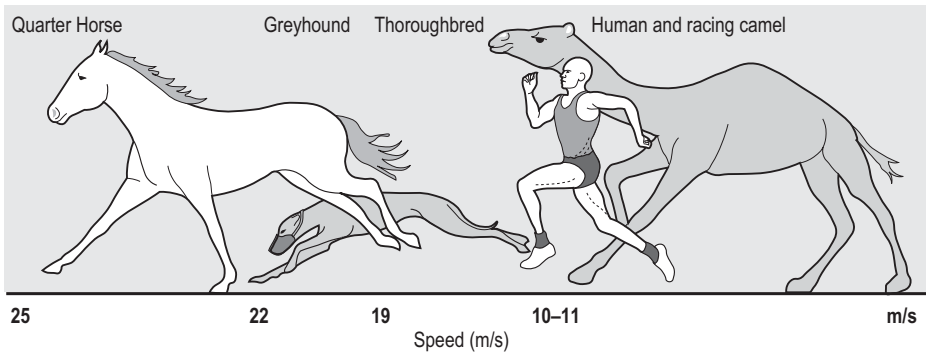
**Fig. 4.1.2**

Chaldean pedigree chart (circa 4000 BC) demonstrating that selective breeding of horses was practiced at least 6000 years ago. (Reproduced with kind permission from Lyons & Petrucelli⁶ and the World Health Organization, Geneva.)

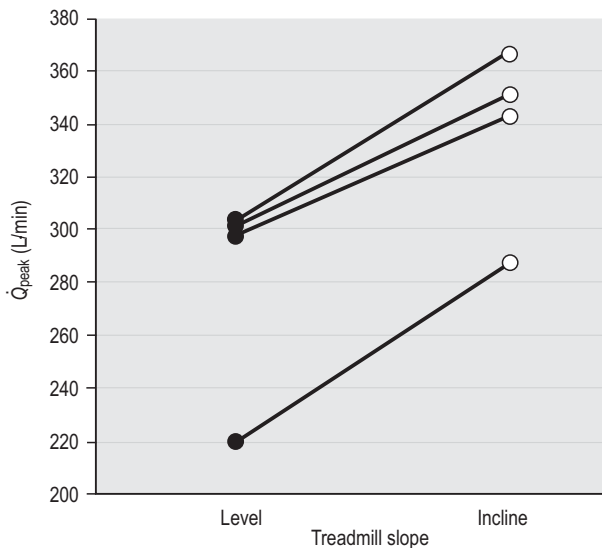
Role of the heart and cardiovascular system in setting aerobic capacity ($\dot{V}O_{2\max}$)

The energetic capability of skeletal muscle is so high that it far surpasses the capacity of the respiratory and cardiovascular systems to deliver O_2 . In Thoroughbreds skeletal muscle comprises over 50% of body mass⁸ and aerobic capacity ($\dot{V}O_{2\max}$) (at least of Standardbreds) increases as a function of fat-free mass.⁹ Moreover, under many conditions $\dot{V}O_{2\max}$ is considered to be O_2 supply-limited because the mitochondrial oxidative enzyme capacity for utilizing O_2 exceeds that of the cardiorespiratory system to deliver O_2 . In support of this notion, there is strong evidence that increasing muscle O_2 delivery during intense exercise will elevate $\dot{V}O_{2\max}$. Specifically:

1. Breathing high O_2 mixtures elevates arterial O_2 content and $\dot{V}O_{2\max}$ in horses¹⁰ and humans.¹¹

**Fig. 4.1.3**

Relative maximum speed in m/s of the four main athletic species. For converting to other commonly used units: 10 m/s = 36 km/h or 22 miles/h. (Revised from Derman & Noakes.⁷)

**Fig. 4.1.4**

Running on an incline (6°) significantly increases cardiac output at maximal exercise ($\dot{Q}_{\text{peak}}$) and also elevates $\dot{V}O_{2\text{ max}}$. (Redrawn from McDonough et al.¹²)

2. With respect to equine $\dot{V}O_{2\text{ max}}$, there is recent evidence that horses run to maximal speeds on an incline (6°) exhibit higher cardiac output ($\dot{Q}$) (Fig. 4.1.4) and $\dot{V}O_{2\text{ max}}$ values than on the flat. This increased $\dot{Q}$ and $\dot{V}O_{2\text{ max}}$ results from an elevated stroke volume¹² and may relate to greater hindlimb muscle recruitment, greater swings in intrapleural pressures (higher tidal volume) aiding heart function, and/or increased hydrostatic pressures improving muscle blood flow.

3. Pericardectomy elevates stroke volume, cardiac output and $\dot{V}O_{2\text{ max}}$ in dogs¹³ and pigs.¹⁴
4. Restricting the exercising muscle mass in humans to 2–3 kg (knee extensors) rather than the 15–20 kg recruited during running or cycling elevates mass specific $\dot{V}O_{2\text{ max}}$.^{15,16}
5. Blood doping (reinfusion of autologous red cells) elevates $\dot{V}O_{2\text{ max}}$ in humans.¹⁷

Several steps in the pathway of O_2 from the atmosphere to its site of utilization within muscle mitochondria may limit the achievable $\dot{V}O_{2\text{ max}}$.^{18,19} These include O_2 diffusion across the blood–gas barrier in the lungs, conductive transport of O_2 in the blood (cardiac output, $\dot{Q}$, and O_2 concentration, CaO_2), and diffusion of O_2 from the skeletal muscle capillary into the myocyte. The coordinated function of respiratory, cardiovascular, and muscular systems provides for rapid changes in O_2 flux from lungs to mitochondria (Fig. 4.1.5) and in most species, including the horse, human, and dog, the strongest determinant of $\dot{V}O_{2\text{ max}}$ is the capacity of the cardiovascular system to transport O_2 (i.e. $\dot{Q}O_2$, Fig. 4.1.6). However, during maximal exercise in the horse other steps in the O_2 pathway also become limiting, in large part because of the disproportionality between the cardiovascular and respiratory systems. For example, O_2 loading in the lung is impaired and arterial hypoxemia becomes manifested (see ‘Cardiovascular physiology and responses to exercise’ below). In addition, the ability to offload O_2 in the muscle capillary is limited by the finite O_2 -diffusing capacity of skeletal muscle which is determined by the capillary bed and blood flow within those capillaries. Figure 4.1.7 integrates the conductive and diffusive

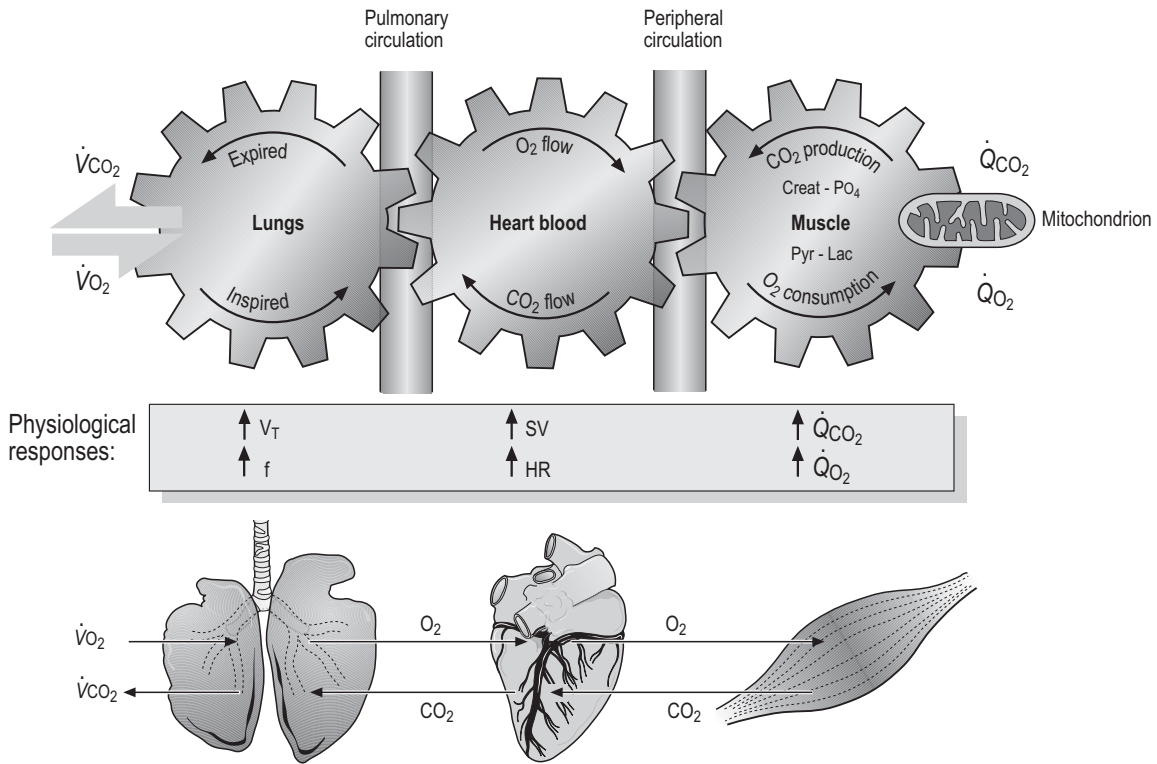
**Fig. 4.1.5**

Illustration of the pathway for oxygen (O_2) from the atmosphere to its site of utilization within muscle mitochondria. The cogs demonstrate that the respiratory (lungs), cardiovascular (heart and blood vessels), and muscle systems must increase O_2 flux in a tightly coordinated fashion for effective delivery of adequate O_2 to facilitate muscular performance. $\dot{Q}_{O_2}$, mitochondrial O_2 delivery; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, CO_2 output; SV, stroke volume; HR, heart rate; V_T , tidal volume; f , breathing frequency; CO_2 , carbon dioxide; Creat- PO_4 , creatine phosphate; Pyr, pyruvate; Lac, lactate; $\dot{Q}_{CO_2}$, mitochondrial carbon dioxide production. (Upper panel redrawn from Wasserman et al.²⁰)

elements of O_2 transport to demonstrate how the horse achieves its very high $\dot{V}O_{2\max}$.

Conductive O_2 transport (lungs to muscle)

Given that high rates of O_2 delivery ($\dot{Q}_{O_2}$) are of paramount importance for achieving this high $\dot{V}O_{2\max}$, it is instructive to break down the components of $\dot{Q}_{O_2}$:

O_2 delivery = cardiac output $\times$ arterial O_2 content

$$\dot{Q}_{O_2} = \dot{Q} \times Ca_{O_2}$$

$$\dot{Q} = HR \times SV$$

Also:

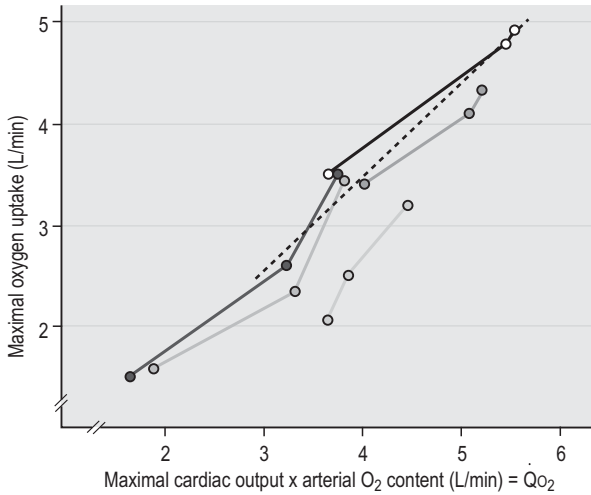
$$Ca_{O_2} = ([Hb] \times 1.34 \times \%Sat) + \sim 0.3 \text{ mL/100 mL dissolved } O_2$$

Therefore:

$$\dot{Q}_{O_2} = (HR \times SV) \times \{([Hb] \times 1.34 \times \%Sat) + \sim 0.3 \text{ mL/100 mL}\}$$

$$\dot{Q}_{O_2} = \text{Heart rate} \times \text{stroke volume} \times [Hb] \times \text{O}_2\text{-binding capacity of Hb} \times \% \text{ of O}_2 \text{ binding sites filled} + O_2 \text{ dissolved in plasma}$$

In the horse during maximal exercise, SV is determined principally by heart size and HR may approach 240 beats/min, which is unusually high for such a large animal. Circulating hemoglobin concentration ($[Hb]$) increases nearly twofold above rest as red blood cells are released from the large muscular spleen in response to increased sympathetic activity. Table 4.1.1 demonstrates that the size of the heart and spleen is relatively larger in the horse than in either the ox or man. Relative heart size in the athletic dog

**Fig. 4.1.6**

Relationship between increased O_2 delivery ($\dot{Q}\text{O}_2$) and maximal oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) after bedrest and exercise training in five humans. (Redrawn from Saltin et al.²¹)

Table 4.1.1 Relative comparison of the weight of organs key to the loading and transport of O_2 as a % of body weight

	Horse	Dog	Ox	Man
Spleen	0.2–1.1	0.3	0.2	0.3
Heart	0.7–1.1	0.8	0.4	0.5
Lungs	0.9–1.5	0.9	0.7	1.4

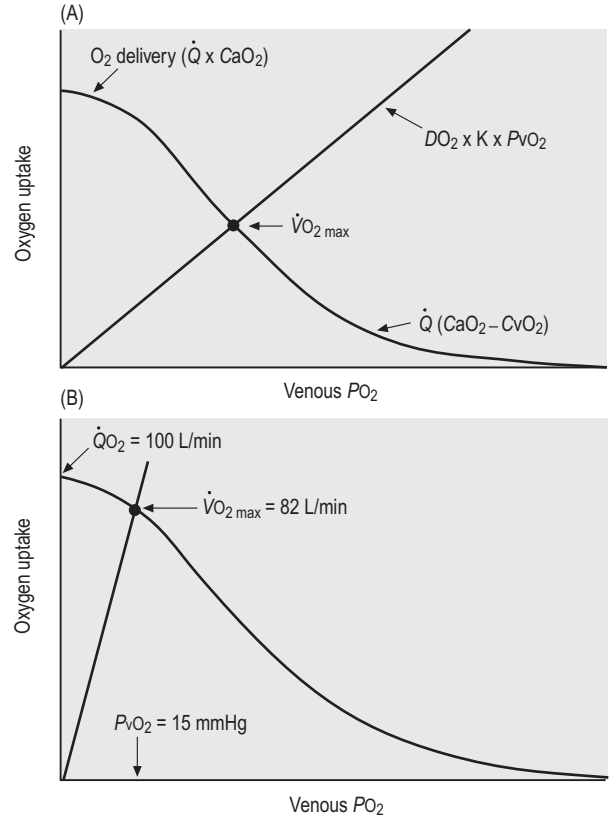
Data from Webb & Weaver.²²

approaches that found in the horse, but the horse is unusual in that the spleen is so large. Note that in the horse, the proportion of skeletal muscle is very high (close to 50%) and the lungs are relatively small (particularly with respect to heart size, see 'Cardiovascular physiology and responses to exercise' below). A comparison between actual heart size and $\dot{V}\text{O}_{2\text{max}}$ in the athletic horse and human is shown in Figure 4.1.8.

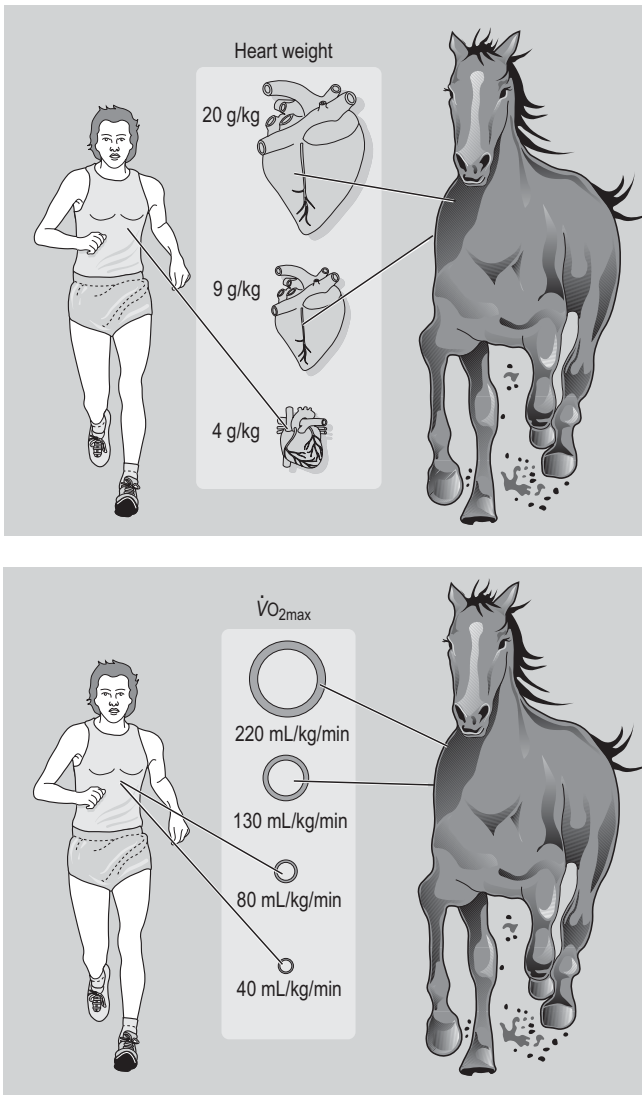
Diffusive O_2 transport within muscle

$\dot{V}\text{O}_{2\text{max}}$ is the product of $\dot{Q}_{\text{max}}$ and the extraction of O_2 primarily by the muscles as described by the Fick equation:

$$\dot{V}\text{O}_{2\text{max}} = \dot{Q}_{\text{max}} \times (\text{CaO}_2 - \text{CvO}_2)$$

**Fig. 4.1.7**

Determination of maximal O_2 uptake ($\dot{V}\text{O}_{2\text{max}}$) by conductive ($\dot{Q}\text{O}_2$) and diffusive movement of O_2 by the cardiovascular and muscle microcirculatory systems ('Wagner' diagram¹⁸). The curved line denotes mass balance according to the Fick principle and the straight line from the origin represents Fick's law of diffusion. DO_2 is effective diffusing capacity and K is a constant that relates venous PO_2 to mean capillary PO_2 . PvO_2 , CaO_2 , and CvO_2 are the partial pressure of venous O_2 and the concentrations of O_2 in arterial and venous blood, respectively. $\dot{V}\text{O}_{2\text{max}}$ occurs at the intersection of the two relationships. (A) is a general schematic whereas (B) presents actual values for a very fit Thoroughbred at maximal exercise. Understanding the conductive and diffusive determinants of $\dot{V}\text{O}_{2\text{max}}$ is essential for interpreting the structural and functional mechanisms that increase $\dot{V}\text{O}_{2\text{max}}$ with exercise training. See text for additional details. ((A) redrawn from Wagner et al.¹⁸)

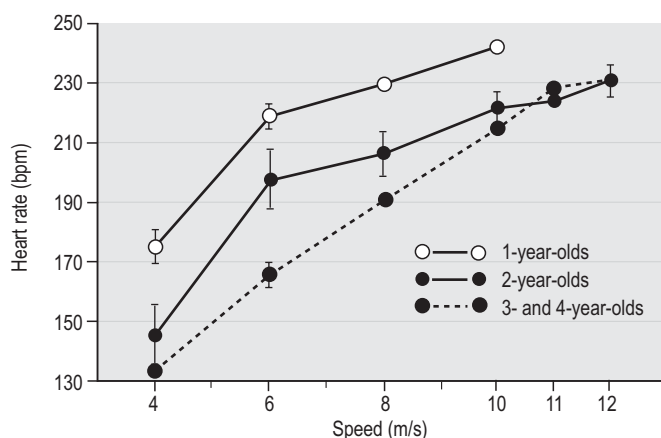
**Fig. 4.1.8**

Comparison of heart size (per kg body weight) and $\dot{V}O_{2\max}$ ranges within healthy human and equine populations. Note that exercise training increases heart size and weight and also $\dot{V}O_{2\max}$ (see Table 4.1.7 for references).

where CaO_2 and CvO_2 denote arterial and mixed venous O_2 contents. Figure 4.1.7 demonstrates that the effective muscle diffusing capacity (estimated by the slope of the line projecting from the origin) determines the level to which CvO_2 will fall at maximal exercise (i.e. extraction) and also the $\dot{V}O_{2\max}$. The section 'Cardiovascular physiology and responses to exercise' details the determinants of muscle diffusing capacity.

This chapter deals primarily with horses in their athletic prime and considers their maximal capacities irrespective of age per se. In this respect, horses increase

their cardiovascular (Fig. 4.1.9) and muscle oxidative enzyme (Table 4.1.2) capacities substantially during their first 3 years. Thus, 3- to 4-year-old horses have larger hearts,^{23,25} and a lower HR²³ (larger stroke volume) at a given running speed, as well as a reduced blood lactate response to submaximal running speeds (Fig. 4.1.10).²³ The mechanistic bases for these last two alterations, as they relate to increased heart size and vascular adaptations which improve muscle O_2 delivery and exchange, are encompassed within the training section of this chapter ('Exercise training').

**Fig. 4.1.9**

Reduction in heart rate response to running as a function of age which reflects increased heart size and stroke volume at 2–4 years of age in comparison with 1-year-olds. (Redrawn from Rose et al.²³)

Table 4.1.2 Citrate synthase (CS), 3-hydroxyacyl-CoA dehydrogenase (HAD), and lactate dehydrogenase (LDH) activities in the middle gluteal muscle of Thoroughbreds and Standardbreds of different ages

		Enzyme activities (mmol/kg/min)							
		Thoroughbreds				Standardbreds			
Age	Sex	No.	CS	HAD	LDH	No.	CS	HAD	LDH
1	M	20	31	20	1793	10	29	29	1936
1	S	21	32	18	1714	15	30	23	1927
2	M	23	44	22	1558	11	35	31	1938
2	S	20	42	22	1458	14	42	25	1639
3	M	21	64	31	1549	12	55	31	1362
3	S	17	58	31	1515	15	54	33	1317
4–6	M	17	67	31	1490	15	56	33	1669
4–6	S	24	67	38	1397	15	68	34	1460
Age			XXX	XXX	XX		XXX	NS	XXX
Sex			NS	NS	NS		NS	NS	NS

Note: Significance over the four age groups: XX, $P < 0.01$; XXX, $P < 0.001$; M, mare (filly); S, stallion (colt).

Reproduced with kind permission from Snow & Valberg.²⁴

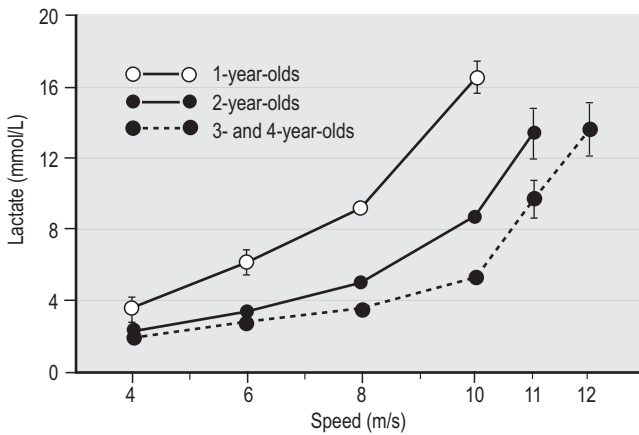
Anatomy of the cardiovascular system

Heart size

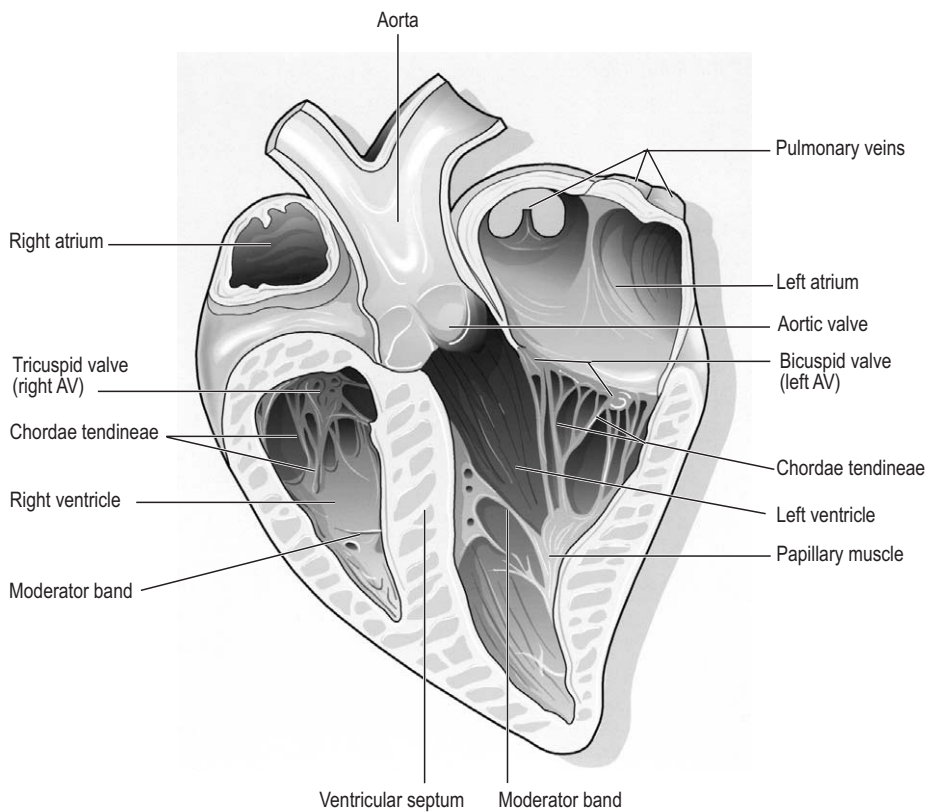
The size of the heart is a key determinant of maximum stroke volume, cardiac output, and hence aerobic capacity and exercise performance (Figs 4.1.11–4.1.13). This relationship has been documented in humans by examination of the electrocardiogram (ECG), ultrasound, radiographs, and post-mortem

examination of heart size. For example, Paavo Nurmi, multiple Olympic champion distance runner reportedly had a heart mass nearly three times larger than predicted for his body size. At post mortem, the heart of the seven-time Boston Marathon winner, Clarence DeMar, who died of a non-myocardial cancer, was substantially larger than normal and his coronary arteries were threefold larger than found in his non-athletic counterparts.²⁷

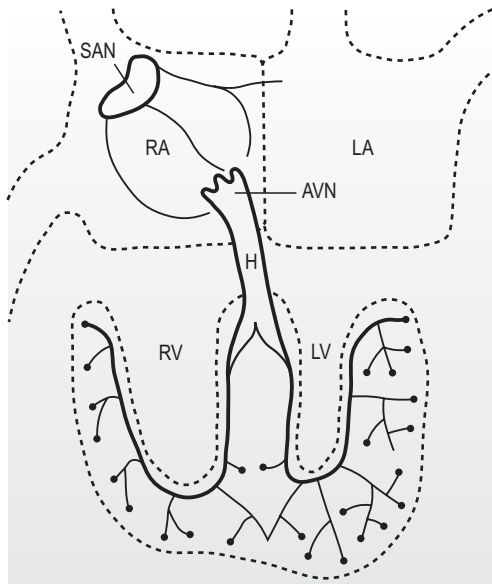
In horses, heart mass approximates 0.9–1% of body mass, which is greater than that for other non-athletic species (Fig. 4.1.14) and may be as much as 1.1% of

**Fig. 4.1.10**

Decreased blood lactate response to submaximal running speeds as horses aged from 1 to 3 and 4 years. (Redrawn from Rose et al.²³)

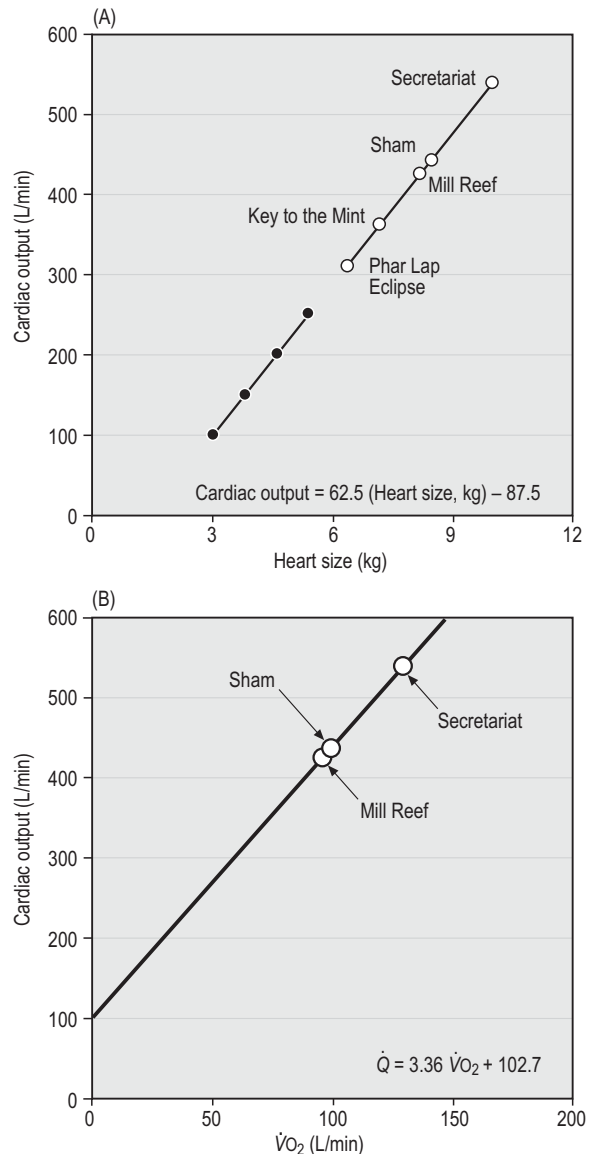
**Fig. 4.1.11**

Cross-section of the equine heart showing principal anatomic structures. AV, atrioventricular valve (see also Fig. 4.1.12).

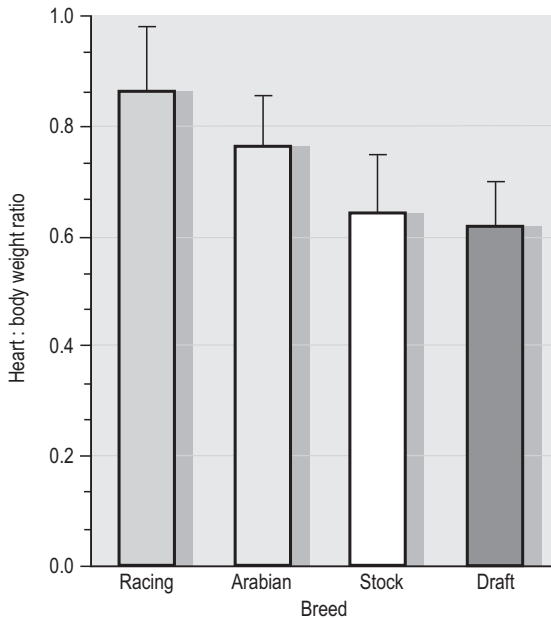
**Fig. 4.1.12**

Conduction system of the equine heart which is composed of specialized cardiac muscle fibers rather than nerves. Note the extensive arborization of the Purkinje fibres across the ventricular walls that is characteristic of the horse heart and which results in effective depolarization from multiple points. SAN, sinoatrial node; RA, right atrium; LA, left atrium; AVN, atrioventricular node; H, bundle of His; RV, right ventricle; LV, left ventricle. (Courtesy of R. Hamlin.)

body mass in trained horses.²² Amongst different horse breeds, racing horses have proportionally larger hearts (Fig. 4.1.14) and Table 4.1.3 lists some famous horses and their heart weights. The heaviest horse heart actually weighed was that of Sham at 18 lb (8.2 kg), who was consistently runner-up to Secretariat. Secretariat was a Triple Crown winner and holds the track record at Belmont Park (2 min 24.4 s for 1.5 miles on turf). Tragically, Secretariat's heart was never weighed. However, the same pathologist, Dr Thomas Swerczek, who weighed Sham's heart, estimated that Secretariat's heart weighed 22 lb (10 kg) and he considered it to be in perfect condition.²⁷ If that weight is correct, Figure 4.1.13A predicts that Secretariat may have achieved a cardiac output in excess of 500 L/min and Figure 4.1.13B a $\dot{V}O_{2\max}$ over 120 L/min! To place heart size in visual perspective, Figure 4.1.15 compares Key to the Mint's 15.8 lb (7.2 kg) heart to that of an unexceptional stallion.

**Fig. 4.1.13**

Relationship between heart size and cardiac output ($\dot{Q}$) (A) and $\dot{V}O_2$ and cardiac output (B) at maximum exercise. (A) The solid symbols are determined from the data of Evans & Rose (1988);²⁶ the hollow symbols are determined from that relationship and the measured or estimated (Secretariat) heart weights published for each named horse.²⁷ (B) An arterial-venous O_2 difference of 22.8 mL/100 mL of blood is assumed to estimate $\dot{V}O_{2\max}$ values for Secretariat and Sham. The echocardiographic data of Young et al⁵ are consistent with this relationship. Note Secretariat's extraordinary cardiac output (~ 540 L/min) and $\dot{V}O_{2\max}$ (over 120 L/min which would be 240 mL O_2 /kg/min at 500 kg bodyweight).

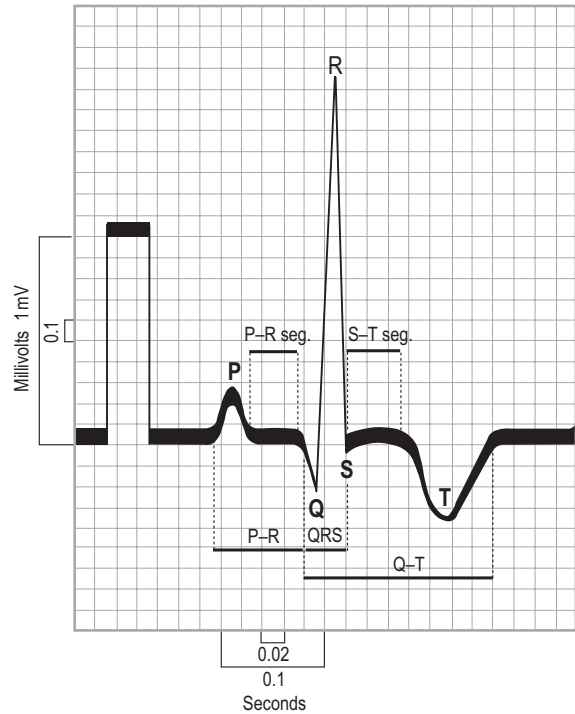
**Fig. 4.1.14**

Heart ratio (heart weight as a percentage of bodyweight) with standard deviations for racing, Arabian, stock, and draft horses. Note that, after training, heart ratio may reach 1.1% or higher.²² (Revised from Kline & Foreman.²⁸)

**Fig. 4.1.15**

Comparison of Key to the Mint's heart on the left (15.8 lb (7.2 kg), heart score 157) with that of an unremarkable stallion on the right (12 lb (5.5 kg)). (Reproduced with kind permission from Haun²⁷ and Dr Thomas Swerczek.)

Given the crucial importance of heart size in setting athletic potential, there has been great interest in establishing a convenient and accurate non-invasive method of estimating this variable in horses. The mean/average duration of the QRS complex (in ECG

**Fig. 4.1.16**

The heart score is calculated from the width of the QRS complex (in milliseconds) on the electrocardiogram shown here. In general, the larger the heart, the wider the QRS complex and the greater the heart score. See text for further details. (Reproduced with kind permission from Haun.²⁷)

leads I, II, and III, Fig. 4.1.16) has been shown to correlate with heart mass at autopsy and also racing performance.²⁸ Thus, larger hearts have a wider QRS complex and the so-called 'heart score' measured in milliseconds has been related to heart mass and subsequently predicted stroke volume and cardiac output, as shown in Tables 4.1.4 and 4.1.5. It should be noted, however, that other studies have not been able to confirm the relationship between heart score and heart mass.^{29–33} Based upon the inheritance of heart scores in race horses,³⁴ there is currently great interest in identifying the gene, located on the X-chromosome, that codes for heart mass.²⁷ Recently echocardiographic and cardiac ultrasound techniques have demonstrated that a significant relationship exists between left ventricular mass and $\dot{V}O_{2\text{ max}}$ in Thoroughbred racehorses.⁵ Moreover, positive relationships among left ventricular size, systolic function, and race performance are emerging.^{35,36} These latter findings offer some hope that non-invasive evaluation methods

Table 4.1.3 Heart weights and heart scores of famous race horses

Name of horse (color, sex, year of birth)	Heart weight		Heart score (ms)
	lb	kg	
Secretariat (ch.s. 1970)	22	10	
Sham* (ch.s. 1970)	18	8.2	
Mill Reef* (b.s. 1968)	16.9	7.7	
Key to the Mint (b.s. 1969)	15.8	7.2	157
Easy Goer (ch.s. 1986)	15	6.8	
Althea (ch.m. 1981)	15	6.8	
Eclipse (ch.s. 1764)	14	6.4	
Phar Lap (ch.g. 1926)	14	6.4	
Star Kingdom (ch.s. 1946)	14	6.4	
Tulloch (b.s. 1954)	13.5	6.2	136
Killaloe (b.m. 1970)	12.9	5.9	
Northern Dancer (b.s. 1961)			150
Soviet Problem (ch.m. 1990)			150
Moscow Ballet (b.s. 1982)			147
The Last Red (ch.m. 1993–twin)			140
Desert Secret (b.s. 1990)			140
Hyperion (ch.s. 1930)			133
Vo Rouge (b.g. 1983)			130

*Some pathologic enlargement (may add 2–3 lb to heart weight).

b, bay; ch, chestnut; g, gelding; m, mare; s, stallion.

Reproduced with kind permission from Haun.²⁷

Table 4.1.4 Relationship derived between heart weight and heart score

Heart score (ms)	Heart weight	
	lb	kg
100	6.6	3.0
110	7.36	3.35
120	10.12	4.6
130	11.88	5.4
140	13.64	6.2
150	15.4	7.0
160	17.16	7.8

Reproduced with kind permission from Haun.²⁷

may prove valuable in helping to select for athletic potential.

An important feature of the equine heart that may contribute to exercise-induced pulmonary hypertension concerns the disparity in size of the right and left atrioventricular (AV) valves. Specifically, at Kansas State University, Professors M. Roger Fedde and Howard H. Erickson (unpublished findings) have determined that the cross-sectional area of the left AV valve is only 63% that of the right AV valve (i.e. 38.1 vs. 60.8 cm²). As the smaller cross-sectional area will substantially elevate resistance to blood flow, this is expected to raise left atrial, pulmonary venous, pulmonary capillary, and, ultimately, pulmonary arterial pressures.

Table 4.1.5 Relationships derived among heart score, heart weight, and stroke volume and cardiac output at maximal exercise

Heart score (ms)	Heart weight (kg) ^a	Stroke volume (L) ^a	Cardiac output (L/min at max exercise) ^b
100	3.0	0.5	100
110	3.8	0.75	150
120	4.6	1.0	200
130	5.4	1.25	250

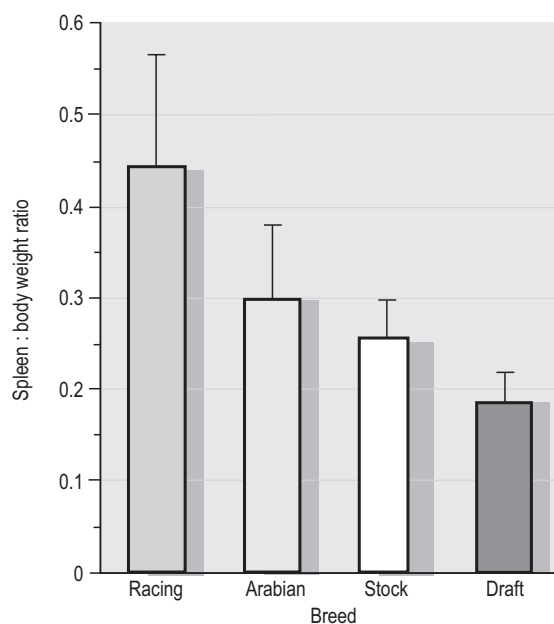
^aEstimated by Steel (unpublished data 1977).^bAssuming a heart rate of 200 beats/min.

Spleen

As shown above, O_2 delivery depends not only upon cardiac output ($\dot{Q}$) but also upon arterial O_2 content (CaO_2), and the equine spleen is of paramount importance for setting the horse's high exercising blood hemoglobin concentration ($[Hb]$) and thus CaO_2 . Splenic contraction may dump 12 L or more of red cells into the circulation, thereby doubling the number of circulating red blood cells.^{37–40} In keeping with the importance of $\dot{Q}_{O_2}$ in determining racing performance, splenectomy reduces $\dot{V}O_{2\max}$ by over 30% in the Thoroughbred.³⁹ Splenic reserve is correlated with spleen weight and total blood volume but not body mass per se.^{32,37,40} Racing horses have a significantly greater splenic mass than non-racing breeds, i.e. Arabian, stock, draft²⁸ (Fig. 4.1.17).

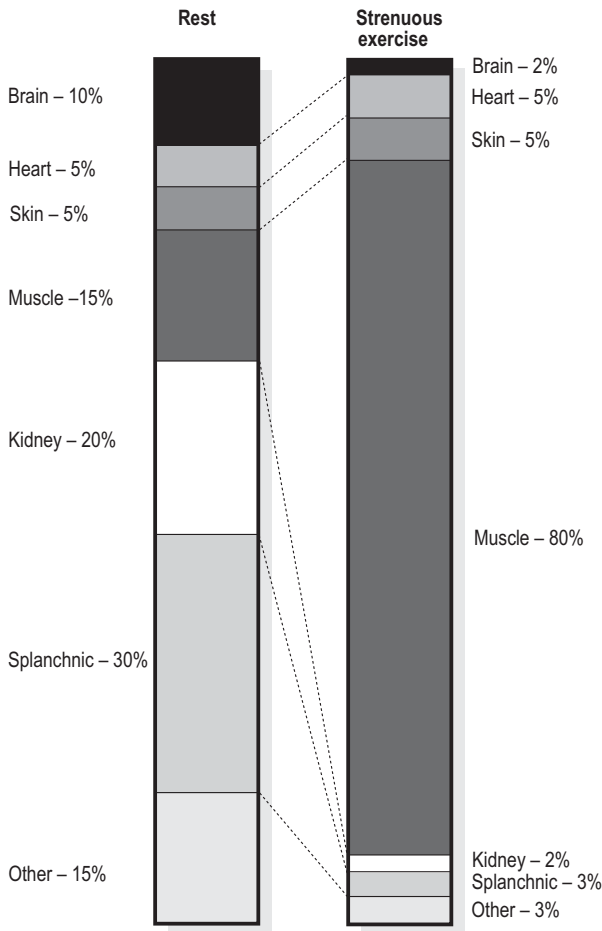
Systemic circulation and microcirculation

In the Thoroughbred, total blood volume approximates 10% of body mass^{41,42} and of this approximately 50 L of blood, 75% resides in the systemic circulation, of which 60% is in the highly distensible venous system and only 15% in the arteries. Depending upon the activity undertaken (e.g. digestion, thermal stress, exercise), the cardiovascular system must redistribute $\dot{Q}$ amongst the appropriate organs. Physical exercise produces the most profound physiological stress to the horse and, as shown in Figure 4.1.18, the percentage of $\dot{Q}$ that perfuses the splanchnic region and kidneys is reduced from ~50% at rest to only 5% at maximal exercise. By contrast, exercising skeletal muscle may receive 80–90% of $\dot{Q}$ compared with only 10–20% at

**Fig. 4.1.17**

Spleen ratio (spleen weight as a percentage of body weight) with standard deviations for racing, Arabian, stock, and draft horses. (Revised from Kline & Foreman.²⁸)

rest. Such flexible and precise control over blood flow distribution demands a network of powerful muscular arterioles that provide the principal resistance to flow in the systemic circulation and which can dilate or constrict rapidly in response to the vasoactive effects of muscle metabolites, prostacyclins, and nitric oxide (dilation), or sympathetic stimulation, angiotensin, and endothelin (constriction). From rest to maximal exercise, skeletal muscle blood flow may increase over 60–70-fold. Although the perfusion pressure does

**Fig. 4.1.18**

Distribution of cardiac output ($\dot{Q}$) at rest and during maximal exercise. In the highly trained, very fit Thoroughbred, it is possible that skeletal muscle blood flow may reach as much as 90% of cardiac output. (Values from Erickson & Poole.⁴³)

increase substantially, elevated muscle vascular conductance (dilation) constitutes the primary mechanism by which the increased muscle blood flow ($\dot{Q}_m$) is achieved, and this response is detailed in the section 'Cardiovascular physiology and responses to exercise' below. Following a systematic series of bifurcations through several orders of progressively narrower arterioles, the vascular tree ramifies into a series of capillaries which form the principal site for blood–tissue exchange (Fig. 4.1.19). The capillary wall is devoid of smooth muscle and presents a barrier typically less than 1 μm thick between the capillary blood and the myocyte sarcolemma. The density, volume, and surface area of the skeletal muscle capillary bed are

correlated closely with oxidative capacity and thus muscle fiber type.^{46,47} In equine muscle (transverse section across fibers), there may be between 400 and 800 capillaries per square millimeter with a mean diameter of 4–6 μm ,^{44,48} and these contain over 80% of the intramuscular blood volume. As we shall see in the section 'Cardiovascular physiology and responses to exercise' below, the capillary volume is crucial for setting red blood cell transit time in the capillary and facilitating O_2 offloading during exercise (or loading in the pulmonary capillary).

Pulmonary circulation and microcirculation

At rest, the pulmonary circulation holds about 20% of total blood volume (mostly in larger compliant vessels rather than the capillaries) which decreases at exercise onset. Because the pulmonary circulation has to accept the whole output of the right ventricle, it is a low-pressure, high-conductance system with arteries and arterioles that are far less muscular than seen for the systemic circulation. Approximately half of the pulmonary vascular resistance is pre-capillary and the capillaries themselves constitute an important site of resistance, particularly at high lung volumes and when alveolar pressure is positive during breathhold or forced exhalation.

The pulmonary vasomotor tone can be influenced by a variety of neural and humoral factors. Specifically, pulmonary arteries have both sympathetic and parasympathetic innervation and respond to serotonin, epinephrine (adrenaline), norepinephrine (nor-adrenaline), isoproterenol, acetylcholine, angiotensin II, leukotrienes, prostacyclins, histamine, thromboxane, bradykinin, and arachidonic acid. However, as seen in Figure 4.1.20, the horse has relatively little pulmonary vascular smooth muscle compared with cattle and pigs.⁴⁹ Thus, the horse exhibits only a weak vasoconstrictive response which is most evident in the low susceptibility of the horse to pulmonary hypoxic vasoconstriction but which causes a profound pulmonary hypertension in cattle and pigs at altitude. During exercise, the elevated pulmonary arterial pressures induce recruitment and distension of the vascular bed which reduces vascular resistance, elevates vascular conductance, and increases pulmonary capillary volume (Fig. 4.1.21). Despite this elevated conductance, pulmonary vascular pressures do become extraordinarily high during maximal exercise (see

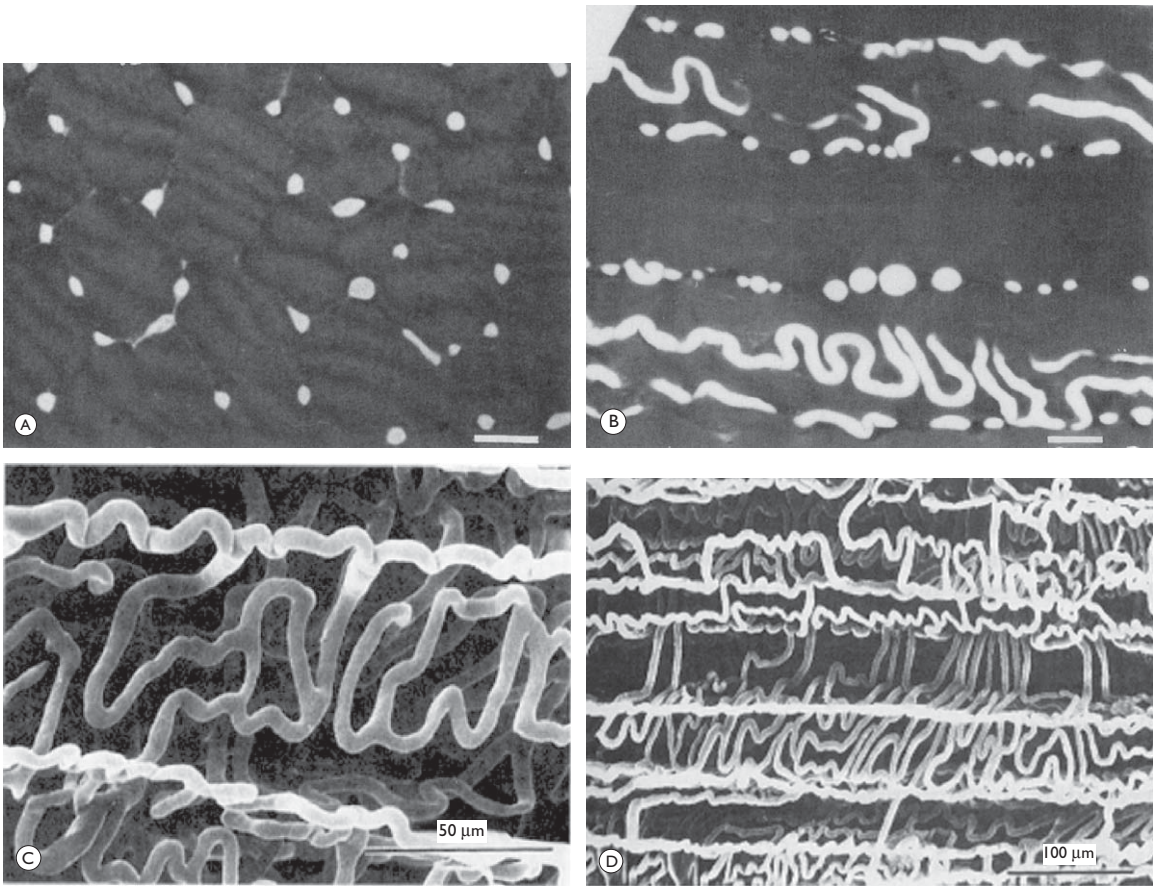


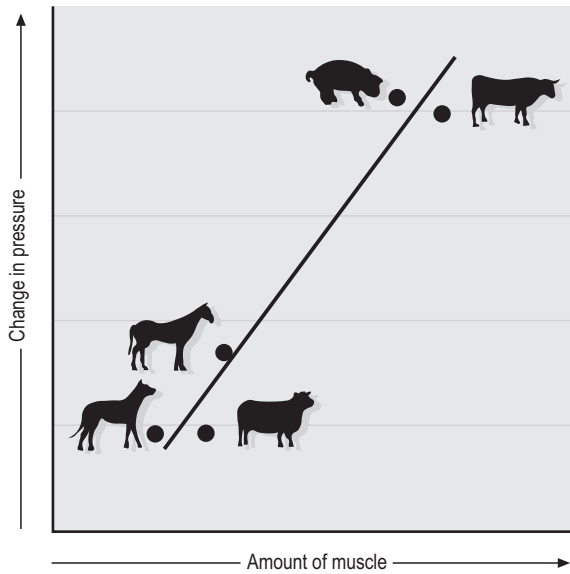
Fig. 4.1.19

The capillary bed of skeletal muscle possesses a complex three-dimensional geometry with extensive branching and capillaries that become extremely tortuous at short muscle sarcomere lengths forming a convoluted network around the fibers. (A, B) Light micrographs of pony vastus medialis muscle perfusion-fixed at 1.90 μm sarcomere length and cut transverse (A) and longitudinal (B) to the fiber longitudinal axis. Capillaries have been flushed clear of red cells and appear white surrounding the perimeter of the individual muscle fibers (A). A, B scale bar = 25 μm . (C, D) Corrosion casts (muscle fibers have been corroded away) of the mouse soleus muscle that demonstrates superbly the three-dimensional geometry of the muscle capillary bed. (A and B reproduced with kind permission from Mathieu-Costello et al.⁴⁴ C and D reproduced with kind permission from Ishikawa et al.⁴⁵)

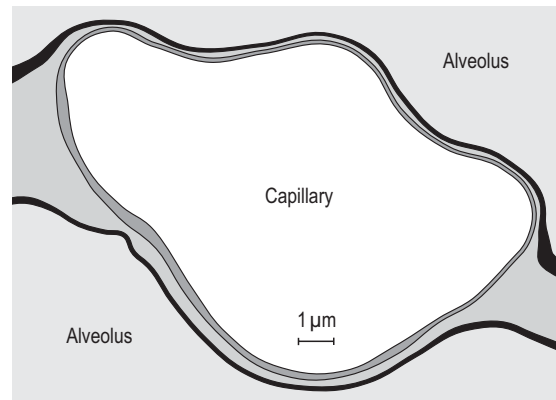
‘Cardiovascular physiology and responses to exercise’ below).

The pulmonary capillaries form a dense plexus around each alveolus and, unlike their systemic counterparts, are not embedded within a supporting tissue matrix and thus are subject to collapse at positive alveolar pressures, particularly when perfusion pressure is low. In the horse, these vessels are about 6–7 μm in diameter⁵¹ (which is narrower than found in dog or rabbit lungs) and have a total wall thickness (capillary endothelium, basement membrane, alveolar epithelium) which averages only 0.9 μm (Fig.

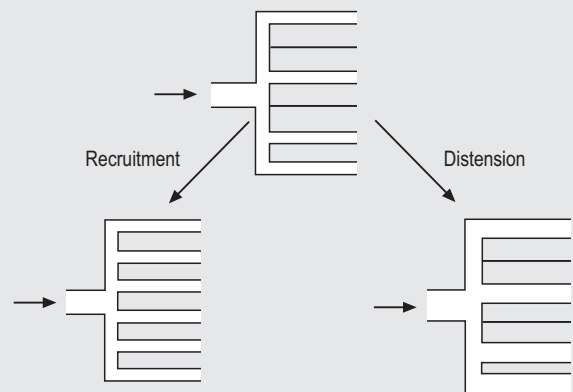
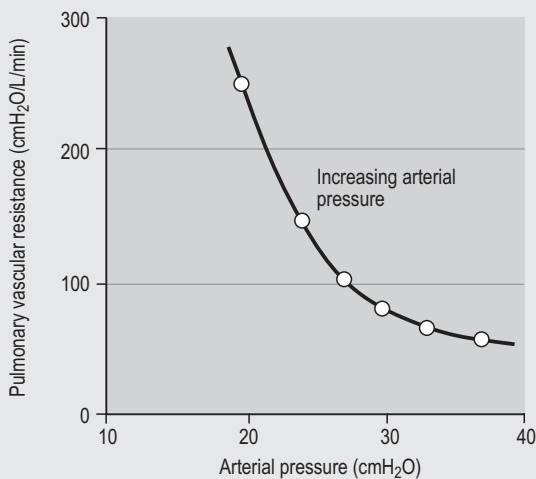
4.1.22). Consequently, even at relatively low transmural pressures (positive luminal plus negative alveolar), these vessels bulge into the alveolar space and may rupture during exercise (exercise-induced pulmonary hemorrhage). Pulmonary capillary blood volume constitutes only a very small fraction of that present in the pulmonary circulation, which confers the advantage of maximizing blood–gas spatial contact but has the consequence of limiting red blood cell transit time in the capillary. Pulmonary capillary blood volume in the horse is 60–80% greater than that of a steer of the same mass.⁵²

**Fig. 4.1.20**

Species variation in the muscle thickness in the walls of small pulmonary arteries and the change in pulmonary arterial pressure evidenced during hypoxic exposure. Note that the horse has far less muscle (and thus change in pulmonary arterial pressure) than the cow and the pig when exposed to hypoxia. (Redrawn from Robinson.⁴⁹)

**Fig. 4.1.22**

Pulmonary capillary interposed between two alveoli. Note the exquisitely thin blood gas barrier. (Redrawn from Birks.⁵¹)

**Fig. 4.1.21**

Elevated pulmonary arterial pressure reduces pulmonary vascular resistance because it forces a recruitment of previously non-perfused vessels and distends those vessels already recruited. (Revised from West.⁵⁰)

In healthy animals, active vasomotor control is thought to play a relatively minor role (compared with the effects of hydrostatic pressure gradients, lung volume, and alveolar pressure) in setting the distribution of blood flow within the pulmonary circulation. However, recent investigations in the horse have demonstrated that there is a gravity-independent distribution of $\dot{Q}$ toward the dorsal aspect of the lung (Fig. 4.1.23).⁵³ This effect may be explained by a regionally

dependent arteriolar responsiveness. For example, arterioles in the upper lung regions exhibit a pronounced vasodilation to methacholine (an endothelium-dependent vasodilator) whereas those at the bottom do not.⁵⁴

Cardiovascular physiology and responses to exercise

Cardiac output

Cardiac output ($\dot{Q}$) is defined as the volume of blood ejected from the right or left ventricle and is usually expressed per minute. As illustrated in Table 4.1.6, cardiac output is the most important means of increasing muscle O_2 delivery during exercise and is the principal determinant of $\dot{V}O_{2\max}$, which can vary from 90 to 220 mL/kg/min^{5,55} or possibly even higher (Secretariat). Very fit Thoroughbreds have had $\dot{Q}$ values measured in excess of 350 L/min and, as mentioned above, based upon estimated heart size, it is likely that superlative athletes have achieved $\dot{Q}$ values between 400 and 540 L/min (Figs 4.1.13A, B, 4.1.14). During submaximal exercise, $\dot{Q}$ (and body O_2 delivery, $\dot{Q}O_2$) increase linearly with running speed and also $\dot{V}O_2$.^{56–58} Increased $\dot{Q}$ in combination with the splenic-induced polycythemia may elevate $\dot{Q}O_2$ over 20-fold in a very fit Thoroughbred race horse from rest to maximal exercise. Increases in $\dot{Q}$ are driven most powerfully by heart rate with a smaller contribution from elevated stroke volume (Table 4.1.6).

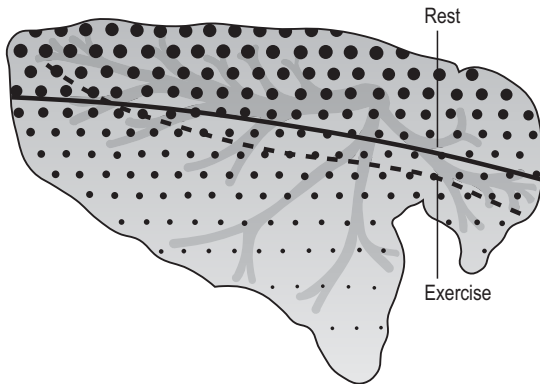


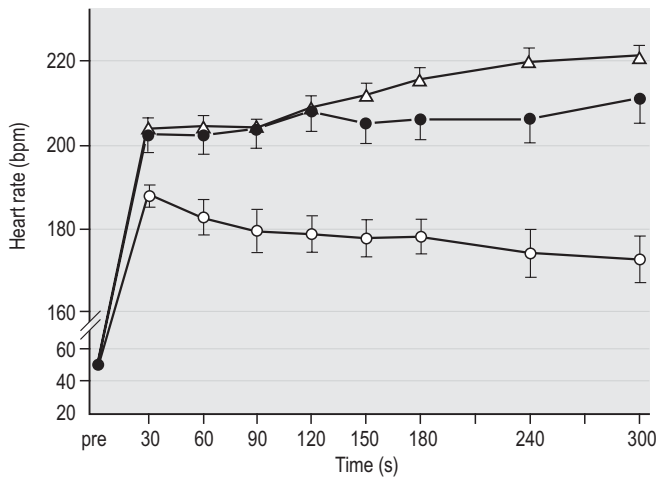
Fig. 4.1.23

Distribution of pulmonary blood flow in the lung at rest. The shading indicates relative blood flow (the darker the area larger dots, the greater the blood flow) and the solid and broken lines indicate relative dorsal–caudal blood flow at rest and during exercise, respectively. Note that pulmonary blood flow is not regulated by gravity, as once thought. (Data from Hlastala and colleagues;⁵³ redrawn from Robinson.⁴⁹)

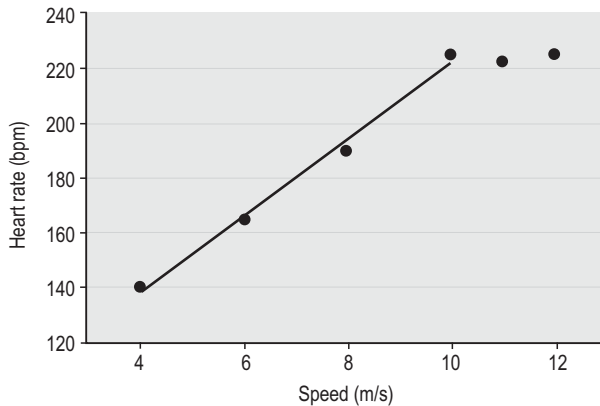
Table 4.1.6 Cardiovascular responses to maximal exercise in a 500 kg horse

Variable	Rest	Exercise	Exercise/rest ratio
Heart rate (beats/min)	30	210–250	7–8
Cardiac output			
L/min	30	240–>450	8–13
SV (mL)	1000	1700	1.7
Systolic/diastolic arterial blood pressure (mmHg)	130/80	230/110	1.6
Pulse pressure (mmHg)	50	120+	3–4
Pulmonary artery pressure (mmHg)	20–30	90–140	3–4
Hemoglobin concentration (g/dL)	13	17–24	1.3–1.6
O_2 consumption (mL/min/kg)	2–4	160–220	40–110
L/min	1.5–2.0	80–110	40–75
Arterial–venous O_2 difference (mL/100 mL)	5	20–25	4–5

Data from Erickson.⁴³

**Fig. 4.1.24**

Heart rate response following the onset of exercise at 50% (hollow circles), 75% (solid circles), and 100% (triangles) $\dot{V}O_2$ max in Standardbred race horses. Note the pronounced early overshoot at 50% $\dot{V}O_2$ max. (Redrawn from Evans & Rose.⁵⁹)

**Fig. 4.1.25**

Relationship between running speed and heart rate from 4 m/s to maximal speed (12 m/s) in a race-fit Thoroughbred race horse. (Redrawn from Evans.⁵⁵)

Heart rate

At exercise onset, heart rate increases rapidly from approximately 30 beats/min at rest to approximately 110 beats/min via parasympathetic withdrawal, with the consequence that at low running speeds heart rate may elicit an early overshoot (Fig. 4.1.24).^{26,59,60} At faster speeds, further heart rate elevations are achieved less rapidly and are driven by the sympathetic nervous system and circulating catecholamines. Maximum heart rate varies between 204 and 241 beats/min and a reduction with age has been described recently in horses.⁶¹ In human populations a decrease of 1 beat/min/yr has been well established.^{62,63} Maximum heart rate is not considered to be an important measure of fitness and, as seen in the section 'Exercise training' below, does not change with training. The speed or velocity a horse can achieve or sustain at a submaxi-

mal heart rate of 140, 170, or 200 beats/min (i.e. V_{140} , V_{170} , and V_{200}) provides information about stroke volume and cardiovascular capacity and pertains directly to fitness and racing potential.⁵⁵ Both heart rate^{26,59} and $\dot{V}O_2$ ⁶⁴ increase faster after a warm-up, although as cardiac output does not appear to limit $\dot{V}O_2$ kinetics at exercise onset,^{65,66} the two are probably not linked mechanistically. There is a linear relationship between heart rate and running speed and between %HR_{max} and % $\dot{V}O_{2max}$ (Figs 4.1.25, 4.1.26).^{55,67–69} However, at a constant running speed in the heavy intensity domain, heart rate may continue to rise and this is accompanied by rising ventilation, $\dot{V}O_2$, and $\dot{Q}$.⁷⁰ This 'slow component' of the cardiorespiratory response is driven by elevated metabolic requirements within the exercising muscles,⁷¹ a progressive fall in blood volume, and also thermoregulatory responses to the rising body temperature.

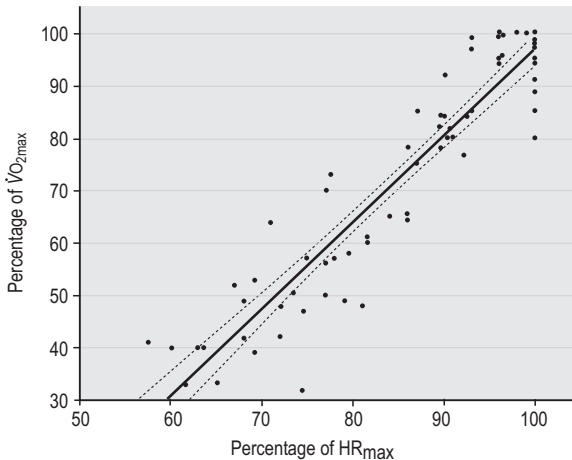


Fig. 4.1.26
Relationship between %maximum heart rate and % $\dot{V}O_{2\max}$ in horses of varying fitness levels. (Redrawn from Evans & Rose.⁶⁹)

Stroke volume

Stroke volume refers to the volume of blood ejected per beat from the left or right ventricle and increases from approximately 1000 mL (2–2.5 mL/kg) at rest up to 1700 mL (3–4 mL/kg) or higher at maximal exercise (Table 4.1.6).^{12,55,56,58,68} If a maximum heart rate of 225 beats/min is assumed for Secretariat, his stroke volume would have been well in excess of 2000 mL/beat. Typically, stroke volume increases sharply at exercise onset up to around 40% $\dot{V}O_{2\max}$ consequent to increased blood volume, venous return, and filling pressures according to the Frank–Starling mechanism.^{26,72} What is particularly remarkable is that ventricular filling (and thus stroke volume) does not appear to be compromised at maximal exercise despite heart rates of 4 beats/s.

Arterial O_2 content (CaO_2) and O_2 delivery ($\dot{Q} \times CaO_2 = \dot{Q}O_2$)

As described in the first section of this chapter, arterial O_2 content, CaO_2 , is determined principally by blood hemoglobin concentration (which sets the O_2 carrying capacity) and the % saturation of those hemoglobin-binding sites with O_2 . At rest, arterial hemoglobin concentration is 12–14 g/100 mL, whereas at maximal exercise the spleen has expelled sufficient red blood cells to increase this up to 21–24 g/100 mL.

This corresponds to a packed cell volume increase from 35% at rest to 70% at maximal exercise. Thus, at maximal exercise, if hemoglobin was 100% saturated with O_2 , each 100 mL of blood would hold between 27 and 31 mL O_2 . However, as discussed below, arterial O_2 saturation falls from around 95% at rest to below 85% at maximal exercise,¹⁰ and this will decrease CaO_2 to 23–26 mL/100 mL. Even considering this effect, with a $\dot{Q}$ of 400 L/min, the Thoroughbred can deliver a prodigious 100 L O_2 /min to the body during maximal exercise.

Determinants of O_2 loading

Pulmonary circulation

The rapid increase in pulmonary blood flow at exercise onset in concert with the polycythemic hyperviscosity^{37,73–75} elevates pulmonary vascular pressures (Fig. 4.1.27) and forces recruitment of non-flowing vessels and distension of flowing vessels (Fig. 4.1.21). This behavior elevates pulmonary vascular conductance several-fold but does not prevent mean pulmonary arterial pressure from exceeding 120 mmHg in fit horses.^{65,76–80} The consequences of this hypertension include exercise-induced pulmonary hemorrhage (EIPH) and are detailed below. Splenectomy (reduced blood viscosity),^{39,75} diuretic therapy (furosemide),^{80–84} and nitric oxide (vasodilator)⁷⁹ are all effective modalities for reducing maximal pulmonary arterial pressures during exercise. However, splenectomy reduces $\dot{Q}O_2$ and thus negatively impacts both $\dot{V}O_{2\max}$ and performance.³⁹ As mentioned in the section, ‘Anatomy of the cardiovascular system’, in contrast to conventional wisdom, it has now been established that gravity is not the sole or even the primary regulator of the regional distribution of blood flow within the equine lung. Elegant fluorescent microsphere studies by Bernard, Hlastala, Erickson, and colleagues have determined that pulmonary $\dot{Q}$ is preferentially distributed toward the dorsal aspect of the lung at rest and during exercise (Fig. 4.1.23)^{53,85} likely due to a regional variation in sensitivity to endothelium-induced vasodilation.⁵⁴

Exercise-induced arterial hypoxemia

As demonstrated in Figure 4.1.28, blood leaving the horse’s lung during maximal exercise is profoundly hypoxemic.^{10,86} Several mechanisms impair O_2 loading in the pulmonary capillary. These are described below in order of importance.

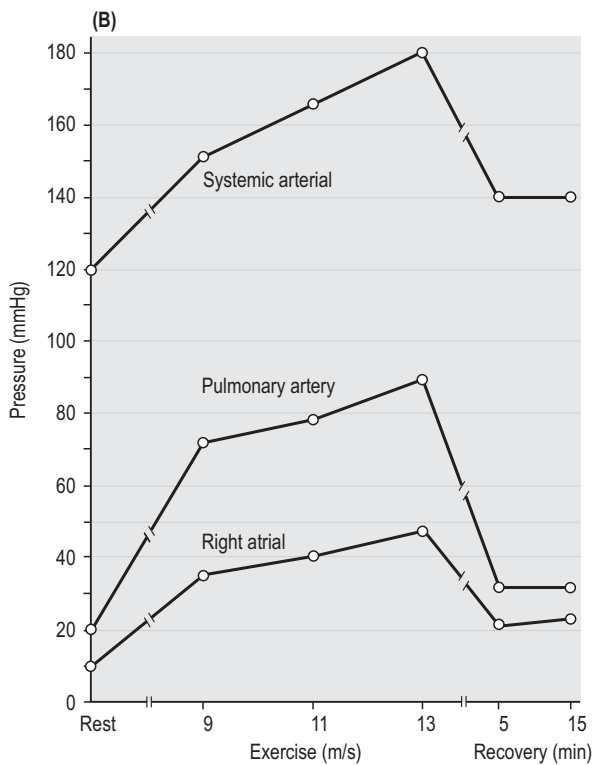
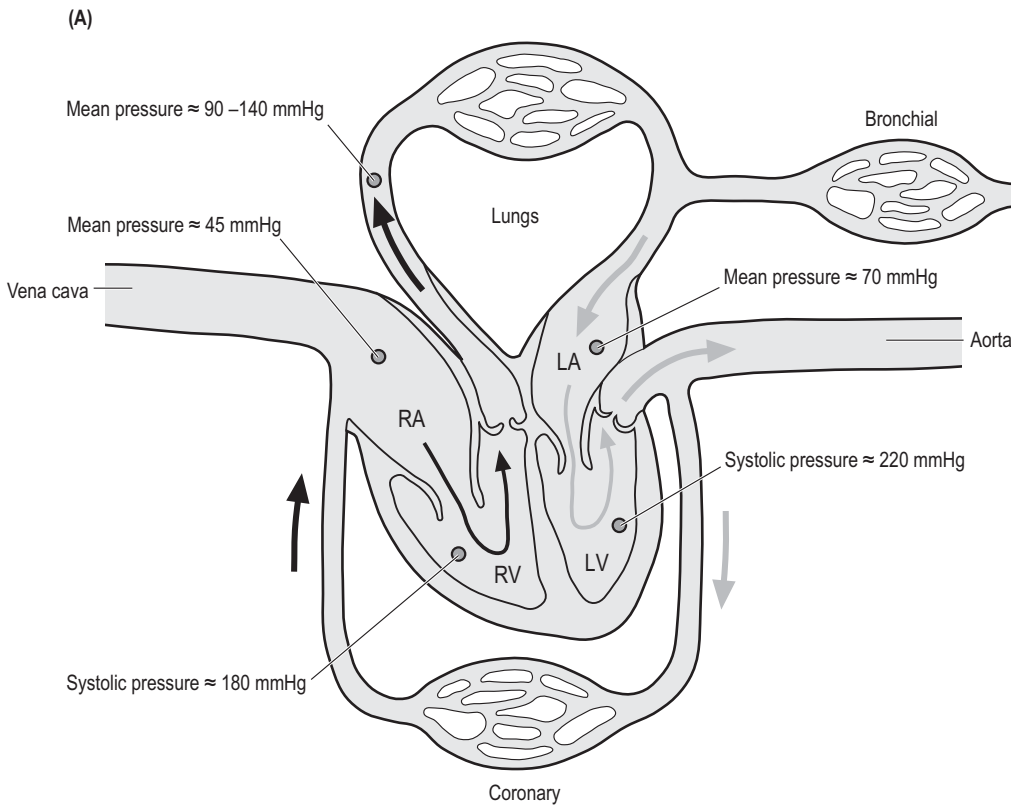


Fig. 4.1.27

(A) Pressures within the pulmonary and systemic circulations during maximal exercise. (B) Mean systemic and pulmonary arterial and right atrial pressures at rest and during exercise and recovery. (Courtesy of S.C. Olsen.)

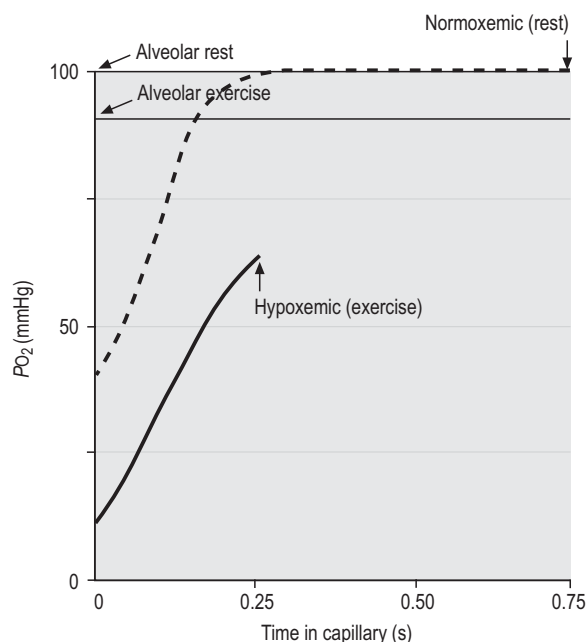


Fig. 4.1.28

Partial pressure of O_2 (PO_2) in the red blood cell as a function of pulmonary capillary transit time at rest and during exercise. Note the reduction in alveolar PO_2 during exercise (alveolar hypoventilation) and the profound decrease in red cell PO_2 as it enters and leaves (arrow on lower curve) the capillary during maximal exercise.

Alveolar–capillary O_2 diffusion limitation (~70% of alveolar-to-capillary O_2 pressure gradient)

During maximal exercise the horse develops a significant alveolar-to-capillary O_2 pressure (P) gradient.⁸⁶ Unlike in other species such as man, in whom alveolar hyperventilation drives arterial PCO_2 below resting and in which alveolar PO_2 becomes elevated during intense exercise, in the horse alveolar PO_2 may fall and consequently the increased alveolar-to-capillary O_2 pressure gradient results from the reduced arterial PO_2 . The primary reason for the elevated alveolar-to-capillary O_2 pressure gradient and arterial hypoxemia relates to the prodigious values of $\dot{Q}$ achieved. The average transit time for a red blood cell (RBC) within the pulmonary capillary is determined by the ratio between pulmonary capillary blood volume and $\dot{Q}$. In the horse at rest, pulmonary capillary volume is some 1.8-fold that of an equivalently sized steer⁵² and RBCs probably spend 0.75–1 s within the capillary, which is thought to be three to four times longer than necessary for equilibration with the alveolar O_2 (Fig. 4.1.28).

However, as we have seen during maximal exercise, $\dot{Q}$ may increase from rest by up to 13-fold and, although capillary volume does increase, it can only do so by a small fraction of this. Consequently, red cell capillary transit time will decrease. Morphometric estimation of mean exercising red cell transit time places it at 0.3–0.5 s.^{52,87} This is probably a gross overestimate of that present in very fit race horses that can achieve $\dot{Q}$ values around 400 L/min and it is pertinent that, even if this were the mean transit time, there would exist a substantial population of red cells with considerably shorter transit times. Because of the sigmoid shape of the O_2 dissociation curve, it is not possible for those cells with longer transit times to compensate for those that do not equilibrate with the alveolar gas. The consequence of this mixing of hypoxemic with normoxemic blood in the pulmonary veins will be arterial hypoxemia. In addition, the rightward shift of the O_2 dissociation curve consequent to elevated blood temperatures, arterial hypercapnia, and acidosis will reduce the hemoglobin– O_2 affinity and further exacerbate alveolar–capillary disequilibrium.

Alveolar hypoventilation

As mentioned above, at maximal exercise the horse's arterial PCO_2 may exceed 65 mmHg.^{10,74,76,88} As calculated from the alveolar gas equation, this will cause alveolar PO_2 to fall from approximately 100 mmHg at rest to approximately 90 mmHg at maximal exercise (Fig. 4.1.28).

Mild ventilation-to-perfusion ($\dot{V}/\dot{Q}$) mismatch

During exercise, the horse develops a small but significant degree of $\dot{V}/\dot{Q}$ mismatch. However, this is not thought to contribute in a quantitatively important fashion to the exercise-induced arterial hypoxemia.^{86,89} The percentage of $\dot{Q}$ that does not come into contact with alveolar gas (i.e. shunt) is trivially small ($\leq 1\%$).

Exercise-induced pulmonary hemorrhage

Exercise-induced pulmonary hemorrhage (EIPH) is characterized by rupture of the blood–gas barrier and the presence of blood in the alveolar space and airways. In extreme cases, frank epistaxis may occur. EIPH is prevalent in Thoroughbreds, Standardbreds, and also Quarter Horses during sprint racing, where repeated endoscopic examination indicates an incidence approaching 95%.^{90–95} Microspheres (10–15 μ m) injected into the jugular vein have identified the pulmonary rather than the bronchial (systemic) vasculature as the site of EIPH⁷⁷ and elegant electron

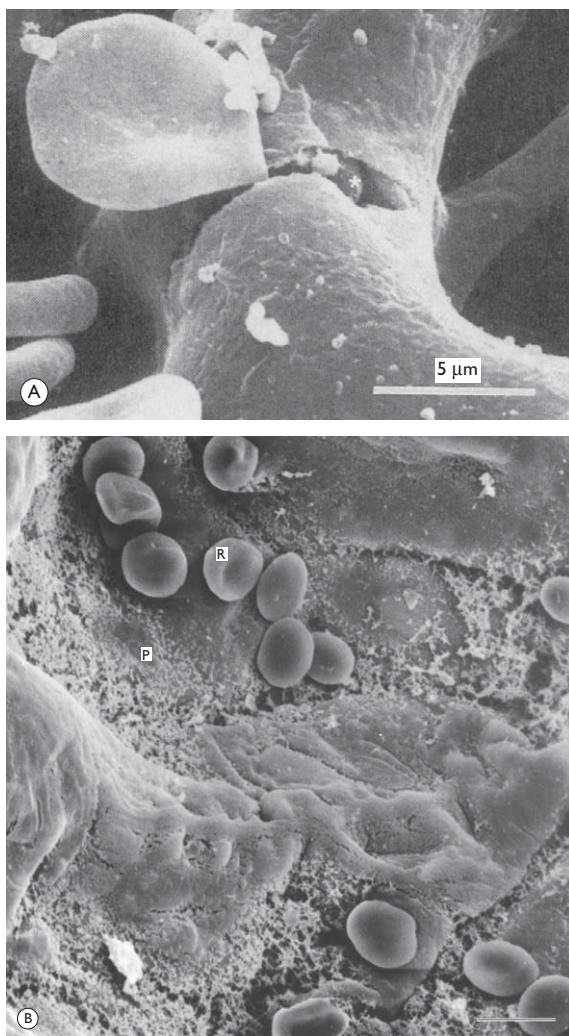


Fig. 4.1.29

Rupture of the pulmonary capillaries. (A) Red blood cell emerging from a split in the blood–gas barrier into the alveolar space. (Reproduced with kind permission from Fu et al.⁹⁶) (B) Exercise-induced pulmonary hemorrhage in the alveolar space of a pony lung. (Reproduced with kind permission from Erickson et al.⁹⁷) R, red blood cell; P, proteinaceous material. Scale bar, 5 μm .

microscopy studies have captured red cells actually erupting from breaks in the fragile blood–gas barrier (Fig. 4.1.29).^{96,97}

EIPH must ultimately arise from high positive intraluminal pressures coupled with very negative alveolar pressures which summate across the blood–gas barrier causing failure (Fig. 4.1.29).^{76,98,99} However, the etiol-

ogy of EIPH is complex and numerous mechanisms have been implicated.⁹⁵ These include:

1. Alveolar pressure fluctuations which may be exacerbated by upper airway obstruction (inspiratory nasal collapse;¹⁰⁰ laryngeal hemiplegia¹⁰¹).
2. Pulmonary hypertension consequent to high $\dot{Q}$ values (>120 mmHg mean pulmonary artery pressure^{76,77,82,99}), exercise-induced hyperviscosity,^{73,102} and possibly arteriolar vasoconstriction.^{74,78,79} In this regard, Thoroughbred horses have higher pulmonary vascular pressures than Standardbred horses and this has been linked to the greater incidence of EIPH in the former.¹⁰³
3. Redistribution of blood within the lung.⁸⁵
4. Mechanical stresses of respiration and locomotion.¹⁰⁴

Additional factors that may contribute to elevated pulmonary arterial pressures include flow limitation induced by the relatively small cross-sectional area of the atrioventricular (AV) valves,⁹⁵ possible regurgitation through the AV valves consequent on high ventricular pressures, and also a left ventricular relaxation rate that may be too slow to allow rapid filling at lower left atrial pressures.^{95,105,106} With regard to valvular regurgitation, Young & Wood¹⁰⁷ performed cardiac auscultation on 111 Thoroughbreds aged 2–5 and reported that the incidence of mitral and tricuspid regurgitation was 7% and 13%, respectively. After training, this increased significantly to 22% (mitral) and 26% (tricuspid).

Following the observation that pulmonary artery pressures and capillary transmural pressures reach extraordinarily high levels in the exercising horse and that there are threshold pulmonary arterial and capillary pressures above which the integrity of the blood–gas barrier is disrupted,^{99,108} EIPH research and therapeutic interventions have focused on reducing these pressures.⁹⁵ Diuretic treatment with furosemide, which lowers pulmonary vascular pressures, has the greatest proven efficacy in this regard.^{80,82,109,110} Nasal passage support with the Flair nasal strip also reduces EIPH, presumably by reducing alveolar pressure swings and the transmural pressure gradient.^{80,100,110,111} Other treatment strategies involving Chinese herbal remedies and IgG are currently being evaluated.¹⁰⁹ For instance, one very recent investigation determined that EIPH was not reduced following herbal supplementation.¹¹² However, preliminary evidence suggests that immunomodulation with concentrated equine serum does decrease the severity of EIPH possibly through a reduction in parenchymal/

airway inflammation and damage.¹¹³ Experiments using furosemide,⁸⁰ nitric oxide inhalation,⁷⁹ and inhibition of nitric oxide synthase^{74,78} have demonstrated that interventions which lower peak pulmonary artery pressure may not necessarily induce a corresponding reduction in EIPH.⁹⁵ Thus, regulation of the distribution of pulmonary $\dot{Q}$ and vascular conductance may be crucial for limiting EIPH and continues to be an active and important avenue of research.

Systemic circulation

Cardiovascular control must subserve two crucial and sometimes conflicting demands. Namely, muscle $\dot{Q}$ must achieve a level commensurate with O_2 and substrate requirements (up to 100-fold resting) whilst maintaining systemic mean arterial pressure (MAP) within acceptable limits. Excessive vasodilation lowers MAP and compromises blood flow to critical organs such as the brain. By contrast, excessive MAP impairs vascular integrity, elevates vascular fluid exudation, and causes tissue damage.

MAP is the product of $\dot{Q}$ and total peripheral resistance (TPR). TPR is determined principally by the aggregate cross-sectional area of all recruited arterioles and also blood viscosity. Figure 4.1.30 details many of the factors controlling vascular smooth muscle and thus vascular conductance within skeletal muscle. Even at maximal exercise, there is a profound sympathetic vasoconstrictor tone within skeletal muscle¹¹⁵ and blood pressure regulation depends upon the interaction of central nervous system reflexes emanating from the brain (central command) and those within the working muscle.^{116–120} MAP rises from 110–138 mmHg at rest to as high as 200 mmHg during maximal exercise (Table 4.1.6, Fig. 4.1.27).^{55,57,74,78,121,122} In addition to MAP, systolic left ventricular and right atrial pressures increase substantially^{55,58,68,77} and myocardial contractility (peak derivative of left ventricular pressure, $LVdp/dt$) rises progressively with running speed.⁵⁵

From rest to exercise, skeletal muscle arterial and arteriolar vasodilation allows TPR to fall precipitously, which facilitates enormous $\dot{Q}$ values with a relatively modest rise in MAP. Specifically, if $\dot{Q}$ increases from 30 (rest) to 450 (maximal exercise) L/min for a corresponding increase in MAP from 120 to 180 mmHg, TPR must fall by 90% (i.e., 4 to 0.4 mmHg/L/min). The control of skeletal muscle vasomotor tone involves

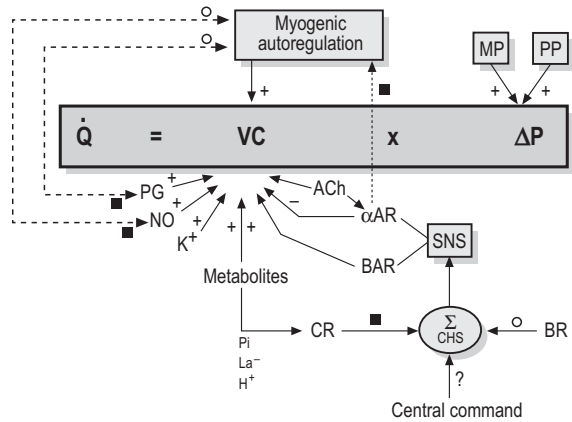
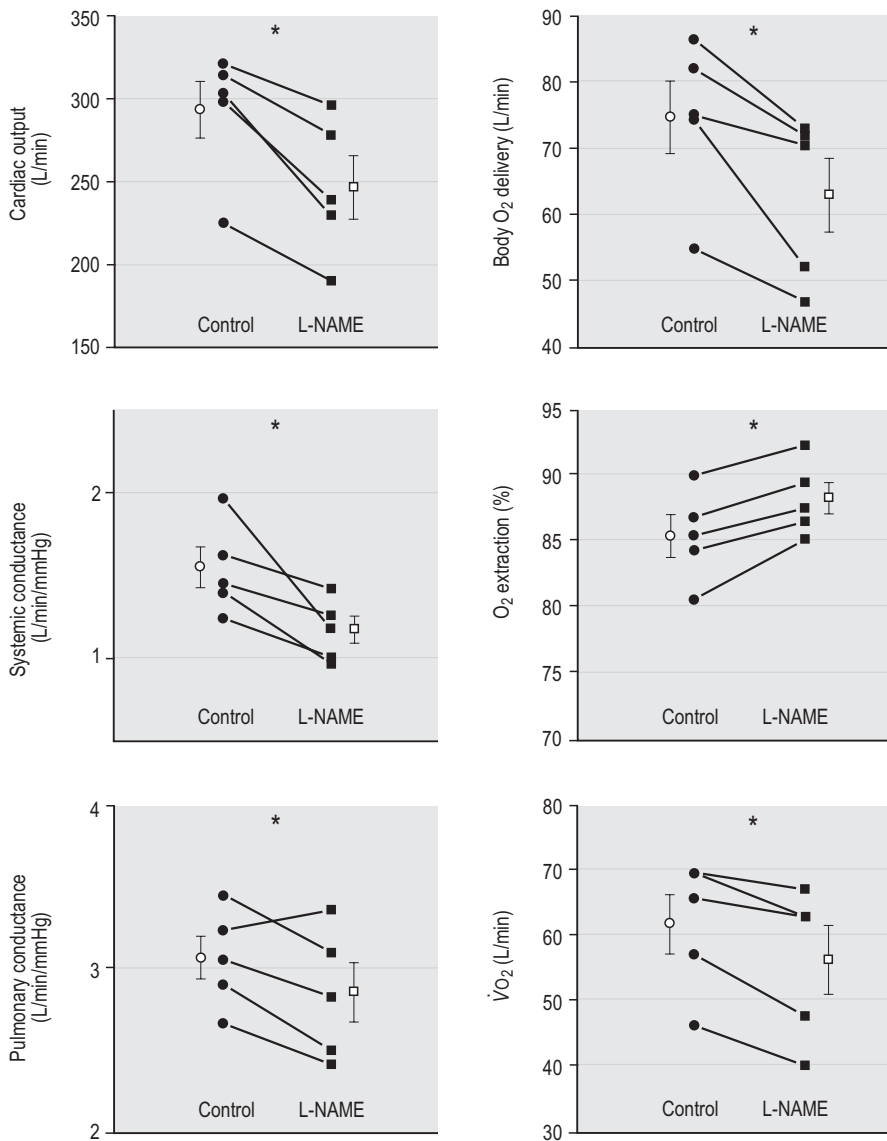


Fig. 4.1.30

Muscle blood flow ($\dot{Q}_m$) is regulated by the interaction of multiple mechanical (top row) and non-mechanical (lower rows) factors which act directly on vascular smooth muscle to either reduce (–) or increase (+) limb vascular conductance (VC). $\dot{Q}_m$ will also increase if the pressure gradient (ΔP) across the capillary bed is enhanced by the muscle pump (MP) following muscle contraction or elevated perfusion pressure (PP). In addition, VC may be changed by the action of the sympathetic nervous system (SNS) after integration of opposing reflex inputs arising from the baroreflexes (BR) and chemoreflexes (CR) and also from higher centers within the brain. There are also other factors that may potentiate (solid squares) or constrain (hollow circles) the effect of other regulatory agents (dotted lines). The net blood flow response to exercise is the result of all of these factors. PG, prostaglandins; NO, nitric oxide; K^+ , potassium ion; Pi, inorganic phosphate; La^- , lactate ion; βAR , beta-adrenergic receptor; αAR , alpha-adrenergic receptor; ACh, acetylcholine. (Redrawn from Shoemaker & Hughson.¹¹⁴)

a complex array of mechanical (muscle pump, shear stress, myogenic), humoral (vasoactive metabolites, catecholamines) and neural (sympathetic, antero-grade and retrograde conducted vasodilation) mechanisms (Fig. 4.1.30). Recent findings from Kindig and colleagues^{74,78,79} indicate a major role for nitric oxide in increasing systemic and vascular conductances during intense running (Fig. 4.1.31). There have been several excellent recent reviews on the regulation of muscle vascular conductance^{118–120,123} and it is apparent that the precise mechanisms which mediate the rapid increase of muscle $\dot{Q}$ and $\dot{Q}_{O_2}$ at exercise onset remain to be resolved.¹²⁴ The matching between $\dot{Q}_{O_2}$ and $\dot{V}O_2$ during exercise is so precise that it has inspired the suggestion that the system behaves as though there is an O_2 sensor located within the muscle or its

**Fig. 4.1.31**

Effect of inhibition of nitric oxide production by L-NAME (nitric oxide synthase inhibitor, N^G -L-nitro-arginine methyl ester) on cardiovascular responses, O_2 delivery ($\dot{Q}O_2$), O_2 extraction and O_2 uptake ($\dot{V}O_2$) at maximum exercise. Solid symbols denote individual horses, hollow symbols are mean $\pm$ standard error. The L-NAME condition significantly ($*P < 0.05$) reduced all variables (except O_2 extraction, which was increased) in addition to the maximum speed attained. (Reproduced with kind permission from Kindig et al.⁷⁴)

vascular bed.¹²⁰ However, if present such a sensor remains elusive.

During exercise, muscle $\dot{Q}$ is distributed heterogeneously between and within muscles depending on their recruitment, oxidative capacity, fiber type, and $\dot{V}O_2$ demands.¹¹⁹ Thus, in the exercising horse, $\dot{Q}$ in heavily recruited, highly oxidative red muscles in the

limbs and respiratory system may achieve peak values of 1–3 L/min/kg.^{48,125–127} Specifically, Armstrong and colleagues⁴⁸ measured vastus intermedius $\dot{Q}$ at 1.5 L/min/kg in Standardbred horses running at $\dot{V}O_{2\max}$ (134 mL/kg/min; $\dot{Q}$ 288 L/min). Within the thigh muscles sampled, the vastus intermedius had the highest citrate synthase activity (mitochondrial

volume density, 9%) and myoglobin concentration, and exhibited the greatest $\dot{Q}$. Indeed, across the several muscles/muscle portions sampled there was a strong correlation ($r = 0.947$) between citrate synthase activity and $\dot{Q}$ at $\dot{V}O_{2\max}$.

The respiratory muscles, and in particular the diaphragm, are extremely oxidative and during near-maximal exercise diaphragm $\dot{Q}$ may exceed 2.5 L/min/kg.^{125,126} Vasodilation and inspiratory resistance studies have revealed that, even at $\dot{V}O_{2\max}$, the diaphragm retains a considerable vasodilator reserve. Specifically, prodigious diaphragm $\dot{Q}$ values close to 4 L/kg/min are feasible.^{125–128} It is quite possible that exceptional athletes or individuals with laryngeal hemiplegia exhibit substantially higher diaphragm blood flows than their less fit but healthy counterparts and that the respiratory muscles ‘steal’ $\dot{Q}$ from the exercising limb muscles,¹²⁹ thereby compromising running performance.

The heart, in keeping with its great energetic demands, rich vascularity, and mitochondrial content,¹³⁰ sustains an extraordinarily high blood flow during exercise. Specifically, in Standardbreds run at $\dot{V}O_{2\max}$, left and right ventricular and septal blood flows increase from their pre-exercise value (0.4–0.6 L/kg/min) up to 2.6–2.9 L/kg/min.^{48,131} Atrial blood flows are somewhat lower at rest (0.3 L/kg/min) and at $\dot{V}O_{2\max}$ (1.3–1.4 L/kg/min). One remarkable feature of the equine heart is that there is little or no gradation of blood flow across the myocardial wall. This is surprising given the high compressive forces to which the subendocardial vessels are subjected as systolic pressures greatly exceed 200 mmHg. There is also evidence that the myocardium retains a substantial vasodilator reserve during maximal exertion, at least in ponies.¹³²

Muscle blood flow ($\dot{Q}_m$) and O_2 delivery ($\dot{Q}_{O_2m}$) across the rest–exercise transition

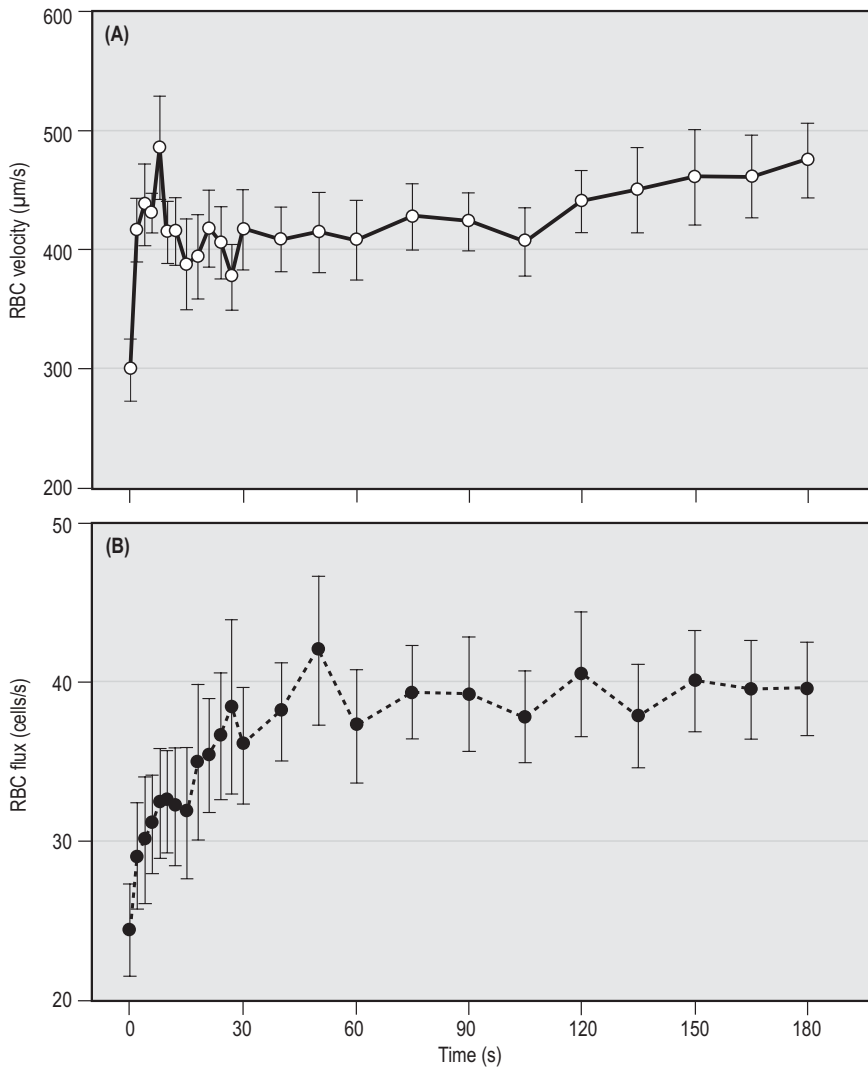
The close matching between $\dot{Q}_{O_2m}$ and $\dot{V}O_2$ (Fig. 4.1.5) presents a strong case for $\dot{Q}_{O_2m}$ being controlled ultimately by muscle metabolism.^{16,118,120,133–136} However, $\dot{Q}_{O_2m}$ increases within the first one or two contractions at exercise onset and this timecourse is generally accepted to be far faster than that of $\dot{V}O_2$;¹³⁷ it is thus too rapid to be explained by metabolic feedback¹³⁶ and arteriolar vasodilation mediated by common vasodilators.¹³⁸ Consequently, it is thought

that the muscle pump, which substantially reduces venular pressure, is key to increasing the pressure differential across the muscle vascular bed and augmenting flow almost instantaneously (Fig. 4.1.32).^{137,139,140} It is also possible that conducted vasodilation, initiated within the capillaries adjacent to active muscle fibers, causes vasodilation upstream within the arteriolar bed.^{123,141} Kindig and colleagues^{65,66} have demonstrated recently that inhibition of nitric oxide formation using L-NAME actually speeds the rate of $\dot{V}O_2$ increase at exercise onset in Thoroughbreds (Fig. 4.1.33). Thus, despite any L-NAME-induced $\dot{Q}_{O_2}$ reduction,^{74,142} relief of nitric oxide-mediated mitochondrial inhibition¹⁴³ allows a more rapid $\dot{V}O_2$ increase. This provides the most compelling evidence to date that muscle O_2 delivery does not limit the energetic ($\dot{V}O_2$) response to exercise.

Determinants of O_2 exchange within skeletal muscle: the microcirculation

In skeletal muscle, O_2 diffuses down its pressure gradient from the capillary towards the mitochondria at a rate ($\dot{V}O_2$) that is determined by the O_2 pressure (PO_2) difference between capillary and mitochondria and the tissue-diffusing capacity for O_2 (D) according to Fick's law ($\dot{V}O_2 = D[PO_2\text{ cap} - PO_2\text{ mito}]$). The enduring dogma that there is a large PO_2 gradient from the myocyte sarcolemma to the most distant mitochondrion is at odds with the more recent observation that intramyocyte PO_2 during exercise is low (1–3 mmHg) and without appreciable transverse or longitudinal variation (Fig. 4.1.34).¹⁴⁴ This is important because it means that the greatest fall in PO_2 occurs in very close proximity (1–2 μm) to the RBC even in the presence of potentially long diffusion distances (>40 μm) to the mitochondrion. Intramyocyte O_2 transport is thought to be facilitated by myoglobin, particularly within fibers in which oxidative enzyme activity is high. The mitochondrial system comprises a catenated network that may enhance O_2 and high-energy phosphate transport.^{145,146}

From the above, the inescapable conclusion is that the size (surface area) and geometry (luminal diameter, tortuosity, branching) of the muscle capillary network combined with the flux and distribution of RBCs within that network are of paramount importance for gas exchange. Within skeletal muscle, capillary surface area is regulated as a function of fiber mitochondrial volume,⁴⁶ which itself is indicative of

**Fig. 4.1.32**

Within the capillary bed of skeletal muscle, red blood cell (RBC) velocity (A) and flux (B) increase within the first few contractions at the start of exercise. (Reproduced with kind permission from Kindig et al.¹³⁹)

maximal O_2 demand (Fig. 4.1.35). Within the major limb muscles there are 700–800 km of capillary length per kg which supply 40–50 mL of mitochondria.^{44,48} The capillaries form a dense, interconnecting network of vessels that average 4–6 μm in diameter (RBCs are 5.5 μm in diameter) that becomes extremely tortuous at short muscle sarcomere lengths.⁴⁴ By contrast, at long sarcomere lengths (>2.8 μm), the capillaries become straight and highly aligned with the muscle fibers. As the capillaries stretch, their luminal diameter is reduced and this increases their resistance to RBC passage.^{147,148}

Observation of capillary RBC flux and distribution during muscle contractions presents a formidable challenge to scientists. It is only very recently that events within muscle capillaries have been observed across the rest–contractions transition. At rest in rat spinotrapezius muscle, approximately 80% of capillaries support RBC flow¹⁴⁸ at a velocity of approximately 250 $\mu\text{m/s}$ and a capillary tube hematocrit that averages only 25–50% of systemic values.¹⁴⁶ The instantaneous muscle O_2 -diffusing capacity is thought to be determined by the number of RBCs in the capillary lying adjacent to the muscle fiber.¹⁴⁹ Consequently,

the total length of capillaries adjacent to a muscle fiber in combination with their hematocrit (i.e. number of RBCs per unit length of capillary) will set the potential for O_2 flux. As muscle contracts and blood flow increases, capillary RBC velocity and flux are elevated rapidly (Fig. 4.1.32) and hematocrit increases towards systemic values.¹³⁹ If we extrapolate what is known from microscopic observations in contracting rodent muscle to the exercising horse using equine capillary morphometric⁴⁴ and blood flow⁴⁸ data, from rest to maximal exercise capillary hematocrit is predicted to increase from approximately 10% up to approximately 60% and mean RBC capillary transit time decreases to about 1–2 s (which is substantially longer than seen in the pulmonary capillaries). This presents a remarkable scenario. Specifically, during maximal exercise, if the systemic polycythemia is expressed at the micro-

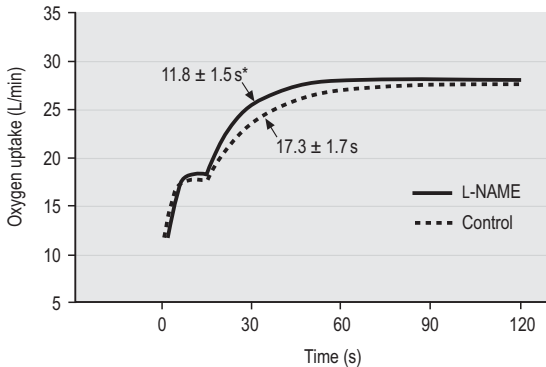


Fig. 4.1.33

Inhibition of nitric oxide production by L-NAME (nitric oxide synthase inhibitor, N^G-L-nitro-arginine methyl ester) significantly speeds the O_2 uptake ($\dot{V}O_2$) response at the onset of moderate speed running (7 m/s). Time constants are given. (Reproduced with kind permission from Kindig et al.⁶⁶)

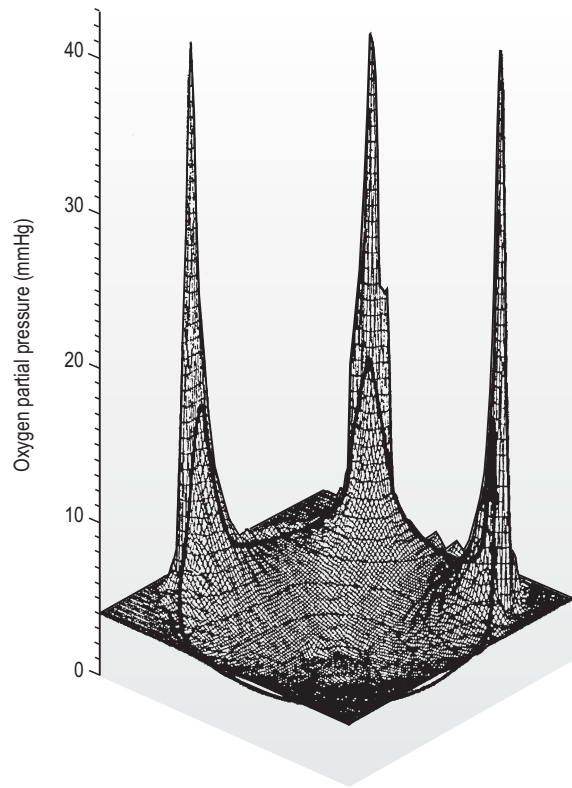
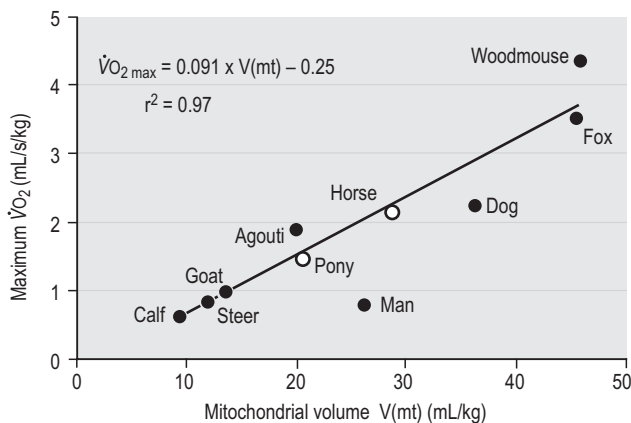


Fig. 4.1.34

Three-dimensional reconstruction of the O_2 partial pressure (PO_2) profile within the muscle capillary and the contracting myocyte. Sarcolemma is indicated by thick line. Notice that the principal drop in PO_2 occurs in close proximity (within 1 μm) to the capillary spikes and that the intramyocyte PO_2 profile is remarkably flat without appreciable PO_2 gradients. This suggests that, at least in red muscle fibers containing myoglobin, even relatively large intracellular O_2 diffusion distances to the mitochondria are of little consequence. (Redrawn from Honig et al.¹⁴⁴)

Fig. 4.1.35

There is a close relationship between whole body mitochondrial content and $\dot{V}O_{2\max}$ across a wide range of mammalian species. This supports the notion that $\dot{V}O_{2\max}$ increases in proportion to the structural capacity for muscle to utilize O_2 . $V(mt)$, mitochondrial volume. (Redrawn from Wagner et al.¹⁸)

circulatory level (as suggested from rodent studies), the sixfold increase in capillary hematocrit will facilitate rapid blood to muscle O_2 exchange. Moreover, as capillary RBC transit time is not thought to become limiting for O_2 offloading until values less than 0.3–0.5 s are reached, the mean transit time of 1–2 s in horse muscle is so long that there likely exists only a small proportion of capillaries where O_2 offloading is limited. These considerations help explain how the horse achieves such excellent O_2 extractions (up to 85–90%) at very high cardiac outputs.

Exercise training

Regular physical exercise or exercise training produces a coordinated pattern of structural and functional adaptations within the cardiovascular and muscular systems. The first three main sections of this chapter dealt with the anatomy and physiology of these systems and their responses to acute exercise (i.e. a single bout). This section details the remarkable plasticity of the cardiovascular and muscular systems in response to training and focuses primarily on those adaptations which elevate $\dot{V}O_{2\max}$ and running performance. There exists a substantial literature on this topic in other species such as the human, dog, and rat,^{47,118,136,150} and this section will avoid an exhaustive overview. Rather it will focus on understanding the mechanistic basis for the elevated training-induced $\dot{V}O_{2\max}$ that occurs in both young and old horses.⁶¹

Historically, $\dot{Q}$ and $\dot{Q}O_2$ have been considered the principal, if not the sole, determinants of $\dot{V}O_{2\max}$ and its increase with training. However, the insightful modeling and novel experiments of Professor Peter D. Wagner at the University of California at San Diego¹⁸ and others^{151–154} have established that $\dot{V}O_{2\max}$ and training-induced increases thereof are the result of a coordinated sequence of adaptations that increases the capacity for conductive and diffusive movement of O_2 from the atmosphere to its site of utilization within muscle mitochondria. The 'Wagner' diagram combines these conductive and diffusive elements (as seen in Fig. 4.1.7), and it is instructive to use this diagram to understand how training increases $\dot{V}O_{2\max}$ (Fig. 4.1.36). The relationship seen in Figure 4.1.36 presents the increased $\dot{V}O_{2\max}$ as an elevated cardiovascular O_2 delivery ($\dot{Q}O_2$) concomitant with an elevated fractional O_2 extraction (increased $CaO_2 - CvO_2$) that reduces muscle effluent and mixed-venous PO_2 . Typically, training-induced increases in fractional O_2

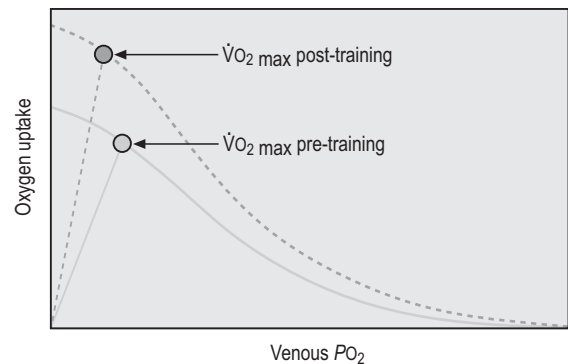


Fig. 4.1.36

The Wagner diagram demonstrates that exercise training increases $\dot{V}O_{2\max}$ by elevating both conductive (curved line, due to increased stroke volume) and diffusive (straight line, due principally to increased muscle capillarity and events within those capillaries) O_2 transport. Note that even a modest reduction in venous PO_2 (5–10%)¹⁵⁵ after training requires a substantial increase (~30%) in muscle O_2 -diffusing capacity. Moreover, if training solely increased convective O_2 delivery and there was no augmentation of muscle-diffusing capacity, venous PO_2 would rise after training. This has not been observed. For additional information see legend to Figure 4.1.7 and Table 4.1.7.

extraction and reduction of venous PO_2 are relatively modest, and this observation has led to the erroneous belief that adaptations to training within muscle that increase O_2 -diffusing capacity (e.g. increased muscle capillarity) are of no, or at least, lesser importance than those cardiovascular changes that elevate $\dot{Q}O_2$. Nothing could be further from the truth. Notice from Figure 4.1.36 that simply increasing $\dot{Q}O_2$ at the same diffusing capacity (i.e. slope of line from the origin to $\dot{V}O_{2\max}$) will decrease O_2 extraction and elevate venous PO_2 . That this response is not seen after training is the result of adaptations within the capillary bed (Table 4.1.7) that substantially increase muscle O_2 -diffusing capacity.^{46,136,156} Table 4.1.7 lists the primary adaptations to training that produce the response evident in Figure 4.1.36.

Training usually increases $\dot{V}O_{2\max}$ between 10 and 25% and, as in other species,^{47,118,136,150} the % improvement is dependent upon initial fitness with fitter individuals having less room for improvement. In addition to an increased muscle O_2 -diffusing capacity, the higher post-training $\dot{V}O_{2\max}$ is driven by an elevated $\dot{Q}$ (and $\dot{Q}O_2$) consequent to an increased SV. Maximal heart rate does not change. At maximal exercise, the

Table 4.1.7 Principal cardiovascular effects of exercise training in the horse at maximal exercise

Variable	% Increase	Reference(s)
$\dot{V}O_{2\text{ max}}$	10–25	69,155,163–166
Mean arterial pressure (at max)	NC	
Total peripheral resistance	Decreased	
Conductive oxygen transport, max		
Heart weight	10+	22,25
Cardiac output, max	Increased	
Stroke volume, max	10+	58
Myocardial hypertrophy		
LV mass	33	25,157
LV internal diameter	7	157
Plasma volume	19–30	155,160,167
Central venous pressure	?	
Pericardial hypertrophy	Yes?	
Systemic [hemoglobin]/hematocrit	NC or decreased	155
Arterial O_2 content	NC or decreased	
Red cell mass	15	155
Heart rate, max	NC	61,163,168
Muscle-diffusing capacity		
Arterial–venous O_2 extraction, max	5	155
Capillarity		
Capillary density	13–36	166,169,170
Capillary–fiber ratio	70	166,171
Myoglobin	?	
Oxidative enzymes	Up to 100	164,169,170,172–174,175
Mitochondrial volume	75–200	166,176
Capillary hematocrit	Increased?	
Capillary RBC transit time	?	
Velocity — submaximal heart rates/blood lactate concentrations		
V_{200}	NC or increased	177
V_{140}	Increased	178
V_{La4}	Up to 31	170,179
La_9	(–51)	179
Run time to fatigue (90–100% $\dot{V}O_{2\text{ max}}$)		
Time	140	166

NC, no change; ?, unknown.

$\dot{V}O_{2\text{ max}}$, maximal oxygen uptake; V_{200} , V_{140} , running velocity at a heart rate of 200 and 140 beats/min; respectively; V_{La4} , running velocity that induces a lactic acidosis of 4 mmol/L; La_9 , blood lactate concentration at a running velocity of 9 m/s.

elevated $\dot{Q}$ occurs without any increase in MAP and so the elevated $\dot{Q}$ must be countered by a precisely matched fall in total peripheral resistance.¹¹⁸

Mechanistic bases for training-induced SV increase

After training, ventricular mass and volume are increased.^{25,157} Training elevates blood/plasma vol-

ume and subsequently end-diastolic volume, and this stretches the myocardial fibers and increases both the force and velocity of contraction. This adaptation occurs in the absence of any substantial changes in myocardial contractility per se^{118,158,159} and, as MAP is unchanged by training, it cannot be caused by modulation of cardiac afterload. Training expands plasma volume^{155,160} and this may elevate ventricular preload by increasing central venous pressure. However, experiments in humans have not demonstrated that expanding plasma volume consistently increases

central venous pressure and SV .¹¹⁸ What is certain is that removal of the pericardium does increase SV and maximal $\dot{Q}$ in the absence of altered preload.^{13,14} Indeed, removing the pericardial constraint to myocardial expansion produces an increased maximal $\dot{Q}$ similar to that found after weeks or months of training. There is evidence that volume overload chronically stretches the pericardial sac¹⁶¹ and decreases its stiffness¹⁶² such that after training a much smaller rise in cardiac filling pressure is needed to increase SV ¹¹⁸ and a greater SV can be achieved. To date, there is no available evidence that pericardectomies have been performed as an ergogenic aid in racing horses. It is pertinent that training-induced increases in heart size may promote valvular (mitral and tricuspid) insufficiencies and regurgitation which will act to limit the improvement in cardiac output.¹⁰⁷

Mechanistic bases for increased muscle vascular conductance and O_2 -diffusing capacity (and increased $CaO_2 - CvO_2$) after training

Arterial O_2 content (CaO_2) is not elevated by training (although small increases in arterial PO_2 during submaximal exercise may be found);¹⁷⁸ hence any increased $CaO_2 - CvO_2$ must result from a decreased CvO_2 . This decreased CvO_2 does not arise from a greater vasoconstriction in other organs or inactive muscle vascular beds but rather from a preferential redistribution of the training-induced $\dot{Q}$ increase towards the active muscles^{118,119,180} and a greater total and fractional extraction of O_2 within those muscles.

Exercise training induces a rapid and profound growth of arterioles and capillaries within skeletal muscle^{47,181–184} and increases the sensitivity of cardiac¹⁸⁵ and skeletal muscle^{186,187} arterioles to vasoactive mediators such as prostaglandins, catecholamines, and nitric oxide. Moreover, training increases the availability of nitric oxide in the myocardium by upregulating endothelial nitric oxide synthase, which is the enzyme responsible for much of the nitric oxide production within the arterial tree.¹⁸⁸ It is quite possible that this latter adaptation occurs also in skeletal

muscle. Exercise training increases $\dot{Q}$ capacity in muscles composed of both slow- and fast-twitch fibers provided they are recruited during exercise.¹⁸⁹ The distribution of $\dot{Q}$ within and between muscles is altered after training, which may be important for improving the matching of O_2 delivery ($\dot{Q}O_2$) to O_2 utilization ($\dot{V}O_2$).^{119,146} After training, capillary length and surface area per fiber volume as well as capillary surface to fiber surface contact is increased in proportion to the elevated muscle oxidative enzyme capacity.^{46,47,136,182} Such capillary proliferation increases the capillary surface area available for O_2 exchange and by increasing capillary volume may prevent or at least constrain any reduction in capillary RBC transit time that would otherwise result from the elevated muscle $\dot{Q}$. It is pertinent that the effect of training on capillary RBC transit time will depend on the precise proportionality between increased $\dot{Q}$ and elevated capillary volume. Moreover, the effect of training on capillary hematocrit (a key determinant of blood–muscle O_2 movement) is not at present known. What is certain is that muscle O_2 -diffusing capacity increases dramatically with training to facilitate a substantially increased total O_2 extraction (Fig. 4.1.36).

Conclusions

Both the cardiovascular and muscular systems evidence great plasticity. During maximal exercise after training, improved cardiac function elevates total $\dot{Q}$ and muscle $\dot{Q}$ (and $\dot{Q}O_2$). Synchronized muscle vascular and intramyocyte oxidative enzyme proliferation permits trained muscle to accept this increased $\dot{Q}$, elevate O_2 exchange, and facilitate a greater O_2 utilization at maximal exercise (increased $\dot{V}O_{2\max}$). At submaximal running speeds, exercise training speeds $\dot{V}O_2$ kinetics,¹³⁶ thereby tightening metabolic control which reduces glycolysis and glycogen utilization,^{47,150} and elevates stroke volume, thereby lowering heart rate. This in turn may actually reduce the $\dot{V}O_2$ cost of running at submaximal speeds.¹³⁶ The training response may be augmented by hypoxia and is modulated by running speed, frequency, and duration.

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Metabolic responses to exercise and training

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Introduction

The superior performance capacity of horses may have its roots in evolution as the means for a horse to escape its predators, and natural selection has led to the survival of the fastest individuals. Several physiologic factors contribute to the horse's athletic prowess (Fig. 5.1.1). Aerobic capacity, as indicated by maximal oxygen uptake,^{1–3} is more than twice that of elite human athletes. This exceptional oxygen uptake is made possible by a high cardiac output and capillary and mitochondrial density in skeletal muscle, and also by the splenic reservoir of 4–12 L of red blood cells released into circulation at the beginning of exercise. Several studies in horses and other species have shown that the augmentation in oxygen-carrying capacity resultant from increased red cell volume markedly increases aerobic capacity.^{4–6} The horse is also advantaged by high muscle glycogen stores^{7,8} that provide a readily available pool of substrate for adenosine triphosphate (ATP) synthesis, thereby avoiding limitations to performance associated with inadequate substrate supply. In addition, equine skeletal muscle has higher buffering capacity compared to other species,⁹ which allows the horse to tolerate high muscle lactate concentrations during exercise.^{10–12} Not only is the muscle able to tolerate high concentrations of lactate, but also the behavior of equine red blood cells (RBC) appears to differ from that of other athletic species. In horses, but not in other species studied so far, RBC appear to function as a lactate sink which may contribute to the efflux of lactate and protons from contracting skeletal muscle.^{13–16}

Skeletal muscle is well adapted for upregulation of metabolic rate with increasing intensity of exercise. For example, oxygen uptake, which at rest may be as low as 4 mL/kg/min,⁶ increases in exercising horses

up to 160–200 mL/kg/min.^{1–3} In Standardbred horses running at 100% of $\dot{V}O_{2\max}$, total muscle blood flow has been estimated at 226 L/min or approximately 78% of total cardiac output.¹⁷ Therefore, the increase in metabolic rate is even greater in contracting skeletal muscle and oxygen consumption may approach 250 mL/kg muscle per min.¹⁸ This change in oxygen uptake indicates acceleration of aerobic pathways for energy transduction, but during high-intensity exercise the rate of anaerobic metabolism also increases. ATP content in muscle remains nearly constant over a large range of changes in muscle activity,^{19–22} indicating that ATP demand for muscle contraction is balanced by adenosine diphosphate (ADP) rephos-

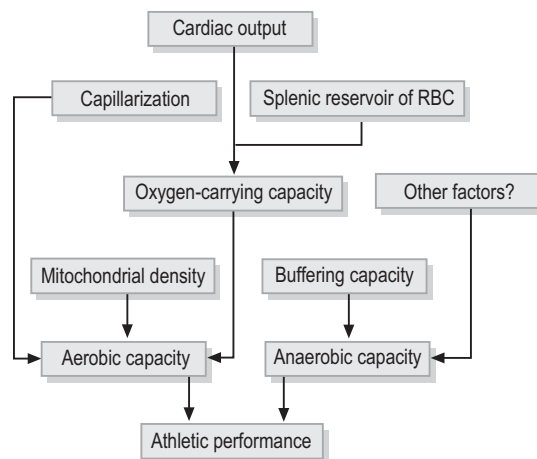


Fig. 5.1.1

Athletic performance from a metabolic viewpoint is a sum of aerobic and anaerobic capacities. When energy sources are not limiting, aerobic capacity depends on both the availability of oxygen and the ability of muscle tissue to use oxygen for energy production. Much less is known about the factors, other than buffering capacity, that influence anaerobic capacity. RBC, red blood cells.

phorylation. During maximal exercise or due to shortage of glycogen and other fuels, the rate of ATP utilization may exceed the rate of production, with resultant activation of the enzyme myokinase and eventually loss of adenine nucleotides. In human skeletal muscle, energy consumption may be up to 3 mmol ATP/kg muscle per s,²³ and, if it is assumed that similar maximal rates occur in equine muscle during intense exercise, it is evident that ATP resynthesis from the aerobic oxidation of glucose or fatty acids will not meet energy demands during maximal exercise.²⁴ The remainder must be derived from anaerobic processes, mainly glycolysis of glucose to lactate.

Integration of various body systems

Exercise requires the coordinated function of the central nervous, respiratory, cardiovascular, hematologic and musculoskeletal systems. At the onset of exercise, the rapid and pronounced effects on the cardiovascular system and overall metabolic rate are initiated and controlled by neuroendocrine mechanisms, with the catecholamines norepinephrine (noradrenaline) and epinephrine (adrenaline) being the primary regulators. These hormones are synthesized and secreted by the adrenal medulla, norepinephrine also by post-ganglionic sympathetic neurons. In horses, high plasma concentrations of epinephrine during exercise suggest that the adrenal medulla plays a more important role in the sympathoadrenal response than in other species.²⁵ Heart rate increases from the resting level of 30–40 beats per minute (bpm) up to 200–250 bpm.²⁶ In addition, cardiac contractility

increases and systole is shortened. Together, these changes result in an up to 10-fold increase in cardiac output, which in horses during exercise may be over 300 L/min, a value that is 2–3 times higher than in steers of the same size.²⁷ Catecholamines also cause contraction of the spleen and thus the release of 4–12 L of RBC into circulation.^{4,28} Due to neural factors, hypoxia, and hormones such as atrial natriuretic peptide, arginine vasopressin and hormones of the renin–angiotensin system, vasodilation occurs in muscles, thereby reducing total peripheral resistance.²⁹ Simultaneous vasoconstriction and reduction in blood flow to other tissues allows blood flow in skeletal muscle to increase by 70–76-fold,²⁶ while an 80% reduction in renal blood flow has been reported.^{18,29–31}

The rate of respiration, which in galloping horses is coupled to stride frequency, also increases. Catecholamines are involved in the stimulation of respiration rate and also contribute to an increase in minute ventilation via relaxation of bronchioles.³²

Hormonal mechanisms are also important for the mobilization and utilization of energy substrates. The catecholamines, together with insulin and cortisol, increase the availability of fuels for energy transduction. In muscle, rapid degradation of glycogen is induced through activation of glycogen phosphorylase and inhibition of glycogen synthesis. Similar changes in the liver lead to release of glucose into circulation, which is further augmented through stimulation of gluconeogenesis. In adipose tissue, catecholamines activate hormone-sensitive lipase, which results in an increase in the plasma concentration of free fatty acids. The inhibitory effect of insulin on lipolysis is attenuated as epinephrine decreases the release of insulin from pancreas (Fig. 5.1.2).

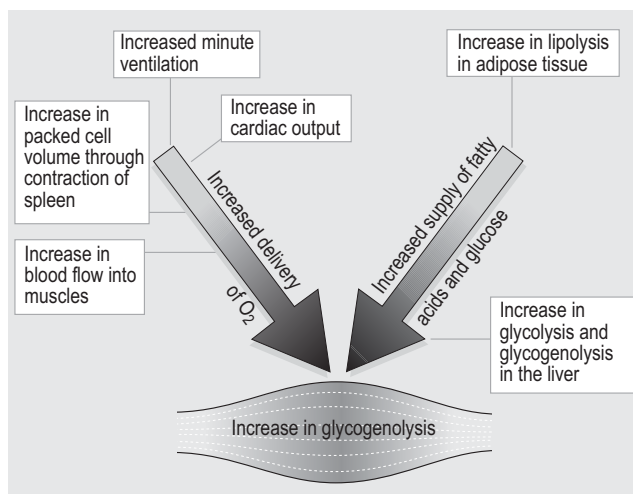


Fig. 5.1.2

Exercise-induced changes in various body systems. Delivery of oxygen to working muscles is increased due to the effects of catecholamines and other hormones on the lungs, heart, spleen, and blood vessels. Simultaneously, catecholamines together with other catabolic hormones increase the supply of energy substrates via circulation and through increased breakdown of muscle glycogen.

Methods of assessing metabolic responses to exercise

Exercise testing

To allow day-to-day or horse-to-horse comparisons, conditions for exercise testing should be standardized, which usually means the use of a high-speed treadmill. Modern, high-speed treadmills are capable of speeds in excess of 17 m/s. However, for safety reasons, exercise tests involving maximal effort are often performed at lower speed with the treadmill set at an incline of up to 6° (10% grade). The advantages of treadmill testing are obvious — the speed and duration of exercise bouts can be controlled and the ambient temperature can be kept constant. Furthermore, in countries where the ambient temperature and humidity are usually low, a climate-controlled treadmill laboratory facilitates exercise testing under conditions of high temperature and/or humidity.^{33–35} Equally important is that while the horse is moving, blood samples can be taken through a catheter placed in a vein or an artery either with a syringe or constantly with the help of a peristaltic pump and a fraction collector.³⁶ Heart rate can be monitored with a pulse meter or by calculating it from an electrocardiogram and activity of different muscles can be recorded by electromyography.³⁷ Furthermore, the horse can be equipped with a mask that allows measurement of respiratory gases. Additional weight may be placed on the horse to simulate the weight of a rider or the horse can actually be mounted by an experienced rider.³⁸ The treadmill is also a suitable tool to study locomotion, because the horse is not moving forward and thus it is possible to film movements with a high-speed camera (Fig. 5.1.3).

Several types of treadmill test have been developed since the 1960s when Professor Sune Persson in Sweden designed a submaximal standardized exercise test,⁴ modifications of which are nowadays frequently used all over the world. In the original test the horse runs four successive 2-minute intervals with the aim of reaching a heart rate of 200 bpm at the highest speed. Blood samples are taken at the end of each 2-min bout that is long enough to allow the heart rate to level off at each speed. This test and its modifications, which include differences in the incline and the duration of exercise intervals, are very useful: for example, for determination of lactate threshold and other indices of aerobic capacity. A typical result



Fig. 5.1.3

A horse running on a high-speed treadmill. (Courtesy of Yrjö Tuunanen.)

from such an exercise test for two horses, one well trained and the other less trained, is shown in Figure 5.1.4.

In tests to determine maximum heart rate and oxygen uptake ($\dot{V}O_{2\text{ max}}$) the intervals are usually shorter, often 1 min, up to a speed when the horse is no longer able to keep up with the treadmill. Treadmill tests are also well suited for endurance-type exercise. The speed may be constant throughout the test or may include faster and slower phases. Either the duration of the test is determined in advance or the test is continued until the onset of fatigue. The test may also be designed to simulate a specific type of event, such as the competition exercise test that simulates the endurance test of a three-day event.³⁹ It is also possible to load the horse with additional weights in a system that simulates draught work.⁴⁰ Because some indices of exercise capacity, such as V_{La4} (the running speed that elicits a blood lactate concentration of 4 mmol/L) or V_{200} (the running speed that elicits a heart rate of 200 bpm), may be influenced by apprehension, the horses should be acclimatized to the treadmill, a procedure that usually takes 1–2 exposures.⁴¹

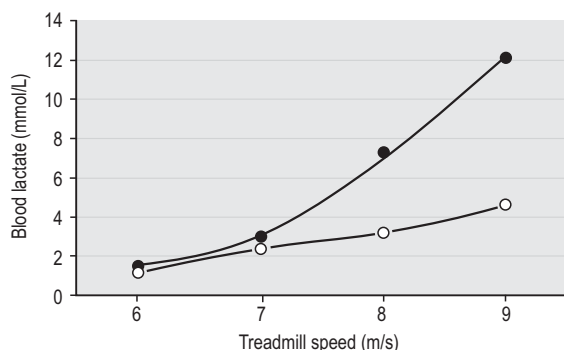


Fig. 5.1.4

Blood lactate response to submaximal treadmill exercise depends on the aerobic capacity of the horse. In untrained horses (filled circles) 2-minute steps at speeds indicated on the x-axis induce a rapid increase in blood lactate concentration, while the higher aerobic capacity of trained horses (open circles) allows energy transduction without accumulation of lactic acid.

The main drawback of treadmill exercise testing is the expensive equipment needed. Therefore horses are often tested on a track, where it is possible to simulate any type of competition but more difficult to control the speed and take blood samples when compared to treadmill testing procedures. Also the testing conditions may vary from one testing occasion to another, because ambient temperature, wind, track surface, and other extraneous factors in the vicinity of the track may influence the results. Furthermore, the horse has to be stopped every time a blood sample is taken and therefore the speed cannot be increased as smoothly as on a treadmill. The stop, although short, may also influence the concentrations of metabolites in blood. Several studies show, however, that track tests are reproducible and reliable,^{42–44} but comparison to treadmill tests is difficult.⁴⁵

Samples and measurements to study the effects of exercise

Blood samples are often used to estimate the effects of exercise, as they are easier to collect and less invasive than muscle samples. From a metabolic viewpoint, the most interesting samples would be those taken from veins coming directly from exercising muscles, especially if combined with arterial samples. These would allow for calculation of oxygen uptake, the consumption of various fuels and the efflux of catabolic products. However, for practical reasons, samples are most

often taken from the jugular vein, which represents mixed venous blood. For some metabolites, the concentrations in jugular venous blood are similar to those in arterial blood,⁴⁶ while others such as lactate differ.⁴⁷ It should, however, be kept in mind that concentration of any substance in blood is a sum of its release into and its disappearance from the blood and the analysis of blood samples gives information only about the very moment the sample is taken, but does not tell anything about the rates of events.

Metabolites, such as non-esterified fatty acids (NEFA), are usually analyzed from blood plasma or serum but for some analyses, especially glucose and lactate, whole blood can also be used. The analysis of lactate is complicated because equine RBC appear to function as a lactate sink and up to 50% of blood lactate may be in RBC.^{13,48} Although there is a close correlation between whole-blood and plasma lactate concentrations,^{13,49} this is not true on an individual basis because the uptake of lactate into RBC varies interindividually.^{15,48} On the basis of lactate transport activity, Standardbred horses can be divided into two populations: one with high and the other with low lactate transport activity in their RBC.^{15,50} Lactate transport capacity appears to be inherited, with the high capacity being caused by the dominant allele.⁵⁰ Because of this interindividual variation in lactate distribution and differences in arterial and venous lactate concentrations,⁴⁷ it is extremely important to standardize the sampling and the treatment of samples when, for example, lactate threshold values are measured. To demonstrate the effects of RBC lactate transport activity on the interpretation of blood lactate data, the following example is given. Two horses had the same blood lactate concentration of 18.7–18.8 mmol/L after exercise. One of the horses with high lactate transport activity in its RBC had a plasma lactate concentration of 21.4 mmol/L, while the other with low lactate transport activity in RBC had a plasma lactate concentration of 29.6 mmol/L. Thus, if lactate threshold were calculated on the basis of blood lactate concentrations, the two horses would have had the same value, but if the determination were based on plasma values, a significant difference would have been recorded.²⁴

As stated above, the blood samples are not suitable for measuring rates or fluxes. To study these, isotope techniques are available. When a small amount of a substrate labeled with a radioactive, e.g. ¹⁴C, or a stable, non-radioactive, e.g. ¹³C, isotope is infused into the circulation, the disappearance of the isotope can

subsequently be analyzed from serial blood samples and the rate of substrate utilization can be calculated. Of these alternatives, the use of stable, non-radioactive isotopes is safer but the disadvantages are the high price of stable isotope-labeled substrates as well as the complexity of the analysis techniques, which also requires special apparatus.^{51–53} Recent equine studies have employed stable isotope tracer techniques in exercise studies. Geor et al,^{54–57} Jose-Cunilleras et al⁵⁸ and Pagan et al⁵⁹ used a constant-rate infusion of a stable isotopically labeled tracer of glucose in order to quantify rates of glucose production and utilization during sustained exertion. From concurrent calculations of whole-body rates of carbohydrate (CHO) oxidation and the rate of plasma glucose disappearance (glucose R_d), it was also possible to estimate the contribution by plasma glucose and intramuscular CHO (glycogen and lactate) to total CHO use. The latter calculations were based on the assumption that the glucose R_d was equal to actual plasma glucose oxidation rate. This assumption has been validated in human subjects during exercise⁶⁰ but not yet verified in horses.

Metabolic changes in muscle are determined by analysis of biopsy samples, which are usually taken from the middle gluteal muscle at the depth of 2, 4, or 6 cm.^{61–65} If samples are taken before exercise and immediately after exercise, it is possible to estimate the consumption of glycogen and accumulation of lactate. Muscle samples are also useful for following the recovery of energy stores and for evaluating training effects, because in addition to the changes in fuels and metabolites, it is also possible to determine the muscle fiber composition, capillarization, and mitochondrial density and to measure activity of enzymes.

Muscle samples can be analyzed as a mixed sample containing all fiber types or on a single fiber or fiber-type basis. For practical reasons most biochemical analyses are performed on mixed muscle samples because of the substantially greater amount of work required for isolation of single fibers. A semiquantitative alternative is the use of histochemical analysis, e.g. for the analysis of glycogen in single fibers. The differences in the results between mixed muscle and single fiber analysis may be enormous. Concerning glycogen analysis, a 50–60% decline may be observed in homogenates of mixed muscle obtained after exercise, but examination of individual fibers from the same sample may reveal complete depletion of glycogen in some fibers and minimal change in others.^{66–69} The same is true for ATP. The concentrations of ATP in some fibers are extremely low after maximal exer-

cise, while in other fibers from the same sample the levels are close to resting values.^{70,71}

Lactate threshold (V_{La4}) and the speed of the horse at a heart rate of 200 bpm (V_{200}) are useful indicators of aerobic capacity and are frequently used in the evaluation of fitness and state of training. These indices are easy to measure, can be determined both on a track and a treadmill, and do not require any expensive equipment. Metabolically, lactate threshold is said to represent the maximal work intensity at which ATP is produced aerobically, i.e. there exists a steady-state situation in blood lactate concentration, wherein lactate is released (mainly from muscles) into circulation at the same rate as it is used by other tissues. Based on studies in humans, it has been shown that blood lactate concentration starts to increase exponentially around a concentration of 4 mmol/L. Similarly, in horses lactate threshold is expressed as the speed of the treadmill or the speed of the horse on a track when blood lactate concentration is 4 mmol/L.

The speed at a heart rate of 200 bpm (V_{200}) has been suggested to indicate both the cardiovascular and metabolic capacities of a horse.⁷² Aerobic capacity, measured as maximum oxygen uptake ($\dot{V}O_{2\max}$), correlates with the running speed over 2000 m.⁷³ $\dot{V}O_{2\max}$ is also useful, because thereafter it is possible to express workloads as a percentage of $\dot{V}O_{2\max}$.⁷⁴ Rose et al⁷⁵ used different exercise protocols to determine $\dot{V}O_{2\max}$ and found that the test itself did not markedly influence the results and the close correlation between maximum oxygen uptake and maximum heart rate allows estimation of the former on the basis of the latter variable.⁷⁶

Anaerobic capacity is far more difficult to estimate and many of the tests used to evaluate anaerobic power of human athletes are not suitable for horses. So far, the only test that can be applied from human sports medicine is the measurement of maximal accumulated oxygen deficit (MAOD), which is calculated from the difference of calculated O_2 demand and maximum oxygen uptake.⁷⁷

The expired gases, oxygen and carbon dioxide, can additionally be used to calculate the respiratory exchange ratio (R), i.e. the ratio of carbon dioxide produced over oxygen consumed. Using stoichiometric equations for the combustion of CHO and fat, values for R can be used to estimate the relative contribution of these substrates to energy expenditure during exercise. It should be noted, however, that these estimates are invalid at high work intensities wherein a portion of expired CO_2 is derived from the bicarbonate

buffer system (the buffering of lactic acid) and values for R may be greater than 1.0.

Sources of metabolic fuel

Fuel for ATP synthesis in skeletal muscle is either stored in the muscle cells or taken up from the circulation. The utilization of fuels is limited by their availability, activity of oxidative enzymes, and the availability of oxygen for their complete oxidation and/or the density of mitochondria in the muscle. Major fuels stored in the muscle fibers are glycogen, triglycerides, and phosphocreatine, and the fuels from circulation are fatty acids and glucose. Because water content of the muscle cells may change during exercise, the concentrations of muscle substrates are often expressed as mmol (or μ mol) per kg dry muscle.

Equine skeletal muscle has a high capacity to store glycogen; in trained horses, typical values are around 600–650 mmol/kg dry muscle.^{66,68,78–80} In human muscle, similar high values are reached only after successful carbohydrate loading procedures. The hydrolyzable carbohydrate content of typical feeds for athletic horses is already high and additional carbohydrate supplementation only slightly increases muscle glycogen content.^{81–83} The content of triglycerides in equine skeletal muscle is highly variable.^{83,84} The third form of fuel stored is phosphocreatine (PCr), a high-energy phosphate. Although the content of PCr is enough to support ATP production for a few seconds only, it is important both in the beginning and at the end of maximal effort.

The main energy stores outside the muscle tissue are adipose tissue for lipids and liver for glucose, the latter derived from hepatic glycogenolysis and gluconeogenesis. During exercise, an increase in epinephrine (adrenaline) concentration contributes to the mobilization of these energy stores. Glucose is released from liver, through the action of glucose-6-phosphatase, and lipids from adipose tissue enter circulation as non-esterified fatty acids (NEFA). Additional glucose may be produced in the liver from glycerol that is a byproduct of lipolysis as well as lactate and alanine formed in the muscle (Fig. 5.1.5).

The release of nutrients from the gastrointestinal tract depends on pre-exercise feeding regimens (see below). In general, equine diets are rich in carbohydrates (e.g. hemicellulose) that are not degraded by salivary and pancreatic amylase, but rather are subjected to bacterial fermentation in the cecum and

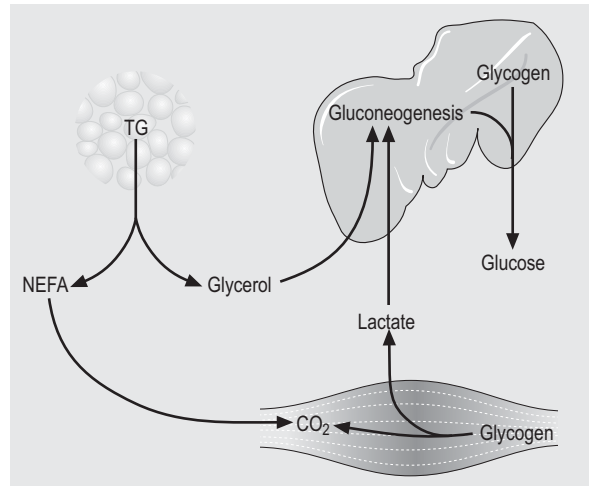


Fig. 5.1.5

During exercise, energy stores in muscle, liver, and adipose tissue are mobilized by the action of catecholamines and other hormones. Fatty acids (NEFA) are released from triglycerides (TG) in adipose tissue and transported by circulation to the working muscles. Muscle glycogen is the body's main store of carbohydrates, but additional glucose from liver glycogen and gluconeogenesis is provided via circulation.

colon. Short-chain (or volatile) fatty acids, mainly acetate, propionate, and butyrate, are produced via this process. It has been shown that, at rest, acetate may provide approximately 30% of the energy utilized by the hindlimb,⁸⁵ while propionate is used mainly for gluconeogenesis.^{86,87} The direct contribution of these substrates to energy use during exercise is not known.

Major metabolic pathways for energy transduction

ATP for muscle contraction may be produced by aerobic and anaerobic pathways. It has to be kept in mind that ATP is not a storage form of energy. Rather, it is the 'metabolic currency' for cellular processes such as muscle contraction and, because cellular stores are small, the rate of ATP synthesis must keep pace with utilization.

Equine skeletal muscle has a high oxidative capacity and, in trained horses, all type I and type IIA fibers are rich in mitochondria, and the activities of oxida-

tive enzymes such as citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HAD) are high.^{88–91} Both carbohydrates and fatty acids can be used as substrates for aerobic energy transduction, the proportion of which varies with the intensity of exercise. During a 400 m Quarter Horse race, about 40% of energy is derived from aerobic metabolism, while aerobic metabolism accounts for 70–80% of the energy used by Thoroughbreds and Standardbreds in races of 1600–2100 m.⁹² During endurance events, nearly all energy metabolism is aerobic.⁹² At rest, when the plasma concentration of fatty acids is low, blood glucose may account for about 80% of oxygen consumed by the hindlimb.⁸⁵ The capacity of aerobic metabolism is limited by oxygen delivery or the capacity of muscle fibers to use oxygen.

The main anaerobic pathway of energy metabolism is glycolysis, for which glucose is derived either from blood or from the glycogen stored in muscle. Although the yield of ATP per one mole of glucose degraded is only 2–3 moles, muscle has a high glycolytic capacity. On the basis of glycolytic enzyme activities measured in equine^{79,90,93} and human²³ skeletal muscle, the capacity for anaerobic energy transduction is well in excess of the maximal rate of energy consumption. The disadvantage of this mechanism is the excessive production of lactate and protons, which eventually will lead to fatigue (Fig. 5.1.6).

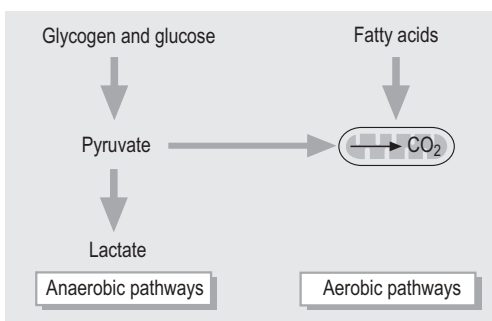


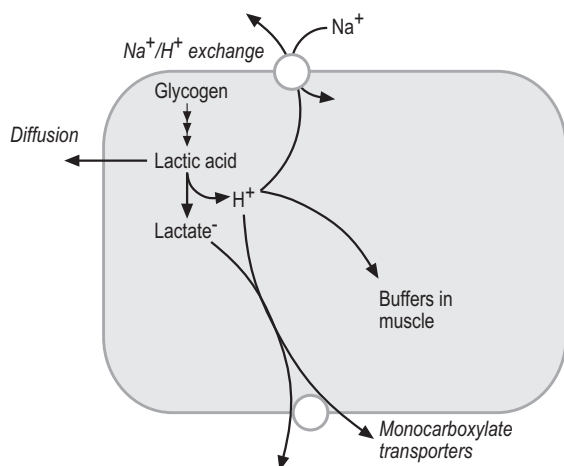
Fig. 5.1.6

The capacity (indicated by the breadth of the arrows) of glycolytic enzymes to produce energy by anaerobic metabolism is high in comparison to aerobic pathways from glucose and fatty acids. The latter are limited by transport of substrates across cell membranes, the number of mitochondria, the activity of mitochondrial enzymes, and the availability of oxygen.

Mechanisms of fatigue

Both high- and low-intensity exercise eventually result in fatigue, but the mechanisms are completely different: the accumulation of lactate, protons, and other metabolic byproducts during intense exercise versus substrate depletion (fuel shortage) during longer-duration exercise at low or moderate intensity. After high-intensity exercise, lactic acid concentrations in muscle range from 100 to 200 mmol/kg dry muscle.^{10–12,19,22,94} The accompanying increase in proton concentration²¹⁸ results in a marked decrease in muscle pH, with values as low as 6.5–6.3 measured in horses following intense, fatiguing exercise.^{19,95} The decrease in pH may be the single most important factor in the development of fatigue during intense exercise. Low pH disturbs calcium release and especially calcium uptake by the sarcoplasmic reticulum, and thus the relaxation phase of muscle contraction cycle is slowed. ATP synthesis is also compromised because the key regulatory enzyme of glycolysis, phosphofructokinase, is inhibited by protons. Furthermore, acidification changes the structure of myosin heads such that the efficiency of ATP binding decreases.⁹⁶ Some recent studies in rat muscle preparations have indicated that acidosis may also be beneficial to the excitability of working muscles through its effect on the function of chloride channels.^{219,220}

The means to prevent intramuscular acidosis include buffers, the most important of which are proteins, the bicarbonate system, phosphate, and carnosine.^{96,97} Although buffering capacity of equine muscle is higher than in other species, it is still not enough to prevent acidosis.^{9,98} The second mechanism for regulation of cell pH is the transport of protons out of the cell.^{99,100} In skeletal muscle, there are two carriers that may facilitate the efflux of protons: the Na^+/H^+ exchange protein and monocarboxylate transporters (MCTs).^{99,100} During Na^+/H^+ exchange, one Na^+ is transported into the cell and simultaneously one H^+ is carried out from the cell. There are no studies on the activity of Na^+/H^+ exchange in equine muscle, but in human muscle this carrier plays a minor role and MCTs, which simultaneously transport one proton together with one lactate anion, are responsible for most proton efflux.^{99,100} Several isoforms of MCTs have been characterized, but only two or three are expressed in skeletal muscle. The main isoforms in muscle are MCT1 and MCT4 and, in some species, MCT2 is also expressed.^{101–103} MCT1 and MCT4 have also been found in equine skeletal muscle.²² In human athletes,

**Fig. 5.1.7**

Lactic acid formed via anaerobic glycolysis is almost completely dissociated into a lactate anion and a proton. The means to prevent acidification of the cell include different buffer systems and transport of hydrogen ions out from the muscle cell. Most hydrogen ions are transported by the monocarboxylate transporter (MCT) with Na⁺/H⁺ exchange playing a minor role.

MCT1, which is the main lactate carrier in oxidative muscles, is upregulated by exercise. On the other hand, the content of MCT4, which is the dominant isoform in glycolytic fibers, increases only with high-intensity training.^{105,106} In human subjects, the endurance training-associated decrease in muscle lactate accumulation during exercise is associated with an increase in skeletal muscle MCT content,¹⁰⁷ demonstrating the importance of MCTs for the regulation of intramuscular pH (Fig. 5.1.7).

During high-intensity exercise acidification may be the main reason for fatigue, but a reduction in energy stores may be another contributing factor. After maximal exercise (e.g. trotting races), some muscle fibers appear to be completely devoid of glycogen and studies of single fibers demonstrate marked ATP depletion.^{66,68,70,71} In rats, there is a linear relationship between ATP degradation in skeletal muscle and the development of fatigue during high-intensity exercise,¹⁰⁸ and depletion of ATP during maximal tests suggests that a similar relationship may also exist in horses.^{11,109}

During endurance exercise, the intensity is usually low to moderate and there is no marked accumulation of lactate.^{67,79,110,111} Therefore, acidification is not a

major cause of fatigue. From a metabolic viewpoint, the major contributing factor to the development of fatigue is a shortage of fuel, especially depletion of glycogen stores,^{79,83} as demonstrated by a correlation between the extent of glycogen depletion and the duration of exercise.¹¹² Low blood glucose concentration also may contribute as it limits the glucose availability to the central nervous system. In addition, central fatigue may be due to increased formation of serotonin in the central nervous system.¹¹³

During endurance exercise, factors not related to substrate supply may be more important in the development of fatigue. These include the loss of electrolytes in sweat which may disturb the neuronal control of muscle contractions, loss of water in sweat that may hamper oxygen and substrate supply via the circulation, and hyperthermia.

Responses and mechanisms

Responses in metabolite concentrations in plasma

During exercise, the major hormonal change in blood is an increase in the concentrations of norepinephrine (noradrenaline) and epinephrine (adrenaline) that, in addition to their effects on the cardiovascular and respiratory systems, induce metabolic changes in muscle, liver, and adipose tissue. The increase in catecholamine concentrations occurs at the onset of exercise and is proportional to exercise intensity. In horses during maximal effort, the responses are several-fold greater than in humans.^{25,114,115} Other hormonal changes with metabolic implications include a decrease in insulin and increases in cortisol and glucagon.^{54,111,116–118}

Due to the above-mentioned hormonal changes, concentrations of metabolic fuels such as NEFA and glucose are increased in circulation. NEFA is released from the adipose tissue together with glycerol. It has been suggested that, of these two, glycerol is a more reliable indicator of lipolysis.^{119,120} The background to this suggestion lies in the fact that muscle tissue, which accounts for about 40% of the bodyweight, is the main user of NEFA and thus even small changes in the oxidation of fatty acids in the muscle will greatly influence the concentration of NEFA in plasma. On the other hand, the concentration of glycerol is not influenced by changes in muscle metabolism as it is mainly utilized by the liver. This is demonstrated by

studies in which NEFA and glycerol concentrations have been measured in tests where the intensity of exercise varies. The results show that NEFA concentration decreases significantly during intense exercise, while glycerol concentration increases steadily.^{80,120,121} Especially during low- to moderate-intensity exercise, there is a progressive increase in lipid oxidation with increasing exercise duration.^{82,122} Horses differ from other athletic animals in that there is an increase in the circulating triglycerides (TG) during exercise.¹²¹ TG are released from the liver as very low density lipoproteins (VLDL),¹²¹ and may have been synthesized in response to increased delivery of NEFA to the liver. Lipoprotein lipase located on the outer side of the endothelial membranes in muscle capillaries will release fatty acids from circulating VLDL-TG for oxidation in muscles.

Changes in blood glucose concentration depend on the type of exercise. Plasma glucose concentration tends to decrease during prolonged exercise (>3 hours), but during short, intense exercise both decreases and increases have been recorded,^{67,118,120,123,124} depending on exercise intensity and training and feeding status of the horse. The regulation of blood glucose during exercise is complex, but changes in prevailing hormone concentrations are important. The exercise-associated increase in hepatic glucose output is mainly mediated via a decrease in the insulin: glucagon ratio, whereas the rate of uptake and utilization in exercising muscle is restrained by increases in circulating epinephrine (adrenaline).⁵⁶

As discussed earlier, lactate is the end-product of anaerobic metabolism and lactate concentrations in blood increase during high-intensity exercise. Lactate

concentrations after different types of competition and exercise test are shown in Table 5.1.1. Usually, the highest lactate concentrations are seen 2–10 minutes after exercise. In equine blood, a significant amount of lactate may be in RBC; uptake is rapid and depends on the activity of MCT on the RBC membrane.⁴⁸

As a result of ATP degradation during high-intensity exercise, breakdown products of purine nucleotides, such as ammonia, hypoxanthine, uric acid, and allantoin, appear in blood.^{14,22,109,127,134} In horses, the predominant end-products of purine catabolism are uric acid and allantoin. The time to peak concentration varies individually and peak concentrations are reached as late as 30 min after exercise.^{134,135} Marked accumulation of uric acid, allantoin, and ammonia is seen mainly after intense exercise, and the breakdown of adenine nucleotides is probably triggered by accumulation of ADP and the decrease in muscle pH.^{127,135,136} In horses, the threshold value for accumulation of these breakdown products is a muscle pH of 6.8, which represents a lactate concentration of 80 mmol/kg dry weight in muscle and about 12 mmol/L in blood.^{22,134} It has been suggested that the peak concentration of uric acid or ammonia is useful as an indicator of ATP degradation in muscles.¹³⁵

Changes in muscle

In muscle, the most prominent changes are a decrease in glycogen content, accumulation of lactate and protons, and degradation of ATP and phosphocreatine (PCr). Glycogen, which is stored as macro- and proglycogen molecules, is the target for glycogen

Table 5.1.1 Maximal lactate concentrations after exercise

	Distance	Blood lactate (mmol/L)	Plasma lactate (mmol/L)	References
Endurance races	80–100 km	1.6 ± 0.1	0.50 ± 0.2	111,116,126
Standardbred races (trotting)	2100 m	21.6 ± 0.9	24.5 ± 0.7	13,91
Standardbred races (pacing)	1760–2160 m		20.9 ± 4.1	130
Thoroughbred races	1100–3800 m	29.6 ± 4.7	33 ± 1.9	47,126–129
Three-day event, cross-country	6200 m		19.1 ± 4.2	126,131
Showjumping			9.0 ± 0.9	132
Polo			9.2 ± 1.2	125
Donkey after exercise			10 ± 1.4	133

Values are means ± standard error of the mean.

phosphorylase that is activated by muscle contraction and epinephrine (adrenaline). From the molecular weight it can be calculated that macroglycogen has about 2100 non-reducing ends from which the release of glucose may begin simultaneously.¹³⁷ Proglycogen, on the other hand, has a much smaller molecular weight and the number of non-reducing ends in this type of glycogen is only 64–128 per molecule.¹³⁷ In humans, proglycogen appears to be more readily available for degradation and in the early phases of exercise, especially when exercise intensity is low to moderate, glycogen breakdown occurs from proglycogen molecules only.^{138,139} In horses during an endurance race, macroglycogen is utilized to a greater extent than proglycogen.¹⁴⁰ Breakdown of macroglycogen is activated only at high-intensity exercise in humans,¹³⁸ but in horses during intense exercise both pro- and macroglycogen seem to contribute equally to glycogenolysis.¹⁴¹ Although the physiological roles and regulation of macro- and proglycogen metabolism are far from clear, subcellular location and the density of branches in the outer tiers of the molecules may be involved.¹³⁹

As mentioned, during fatiguing high-intensity exercise muscle glycogen concentration decreases by up to 50%. Although fatigue during such exercise is often ascribed to accumulation of lactate and protons and depletion of PCr and ATP,^{69,109} depletion of glycogen in individual fibers may also contribute.^{66,69} After endurance rides a more complete depletion of glycogen is seen and, in some horses, muscle biopsy samples may be completely devoid of glycogen.^{67,110}

Marked accumulation of lactate in muscle is seen only when the intensity of exercise exceeds the anaerobic threshold. After a near-maximal exercise test, lactate accumulation in muscle correlates with the amount of glycogen utilized.¹⁴² Accumulation depends also on the oxidative capacity of muscle,^{94,142} and thus horses that have a high proportion of type IIB fibers with low oxidative capacity have higher lactate concentrations in their muscles after high-intensity exercise.⁹⁴ In addition, muscle hemodynamics^{100,143} and the activity of MCT¹⁰⁷ (i.e. the rate of lactate efflux) will influence the extent of lactate accumulation in muscle. Muscle lactate concentrations up to 200 mmol/kg dry weight have been measured in equine muscle.^{12,22}

The changes in muscle TG content with exercise are more difficult to estimate because interindividual variation, both in TG content and utilization rate, is high.^{83,84,142} In middle gluteal muscle, TGs are stored mainly in type I fibers, and type IIB fibers have the lowest content.⁷⁹ A decrease in muscle TG content is

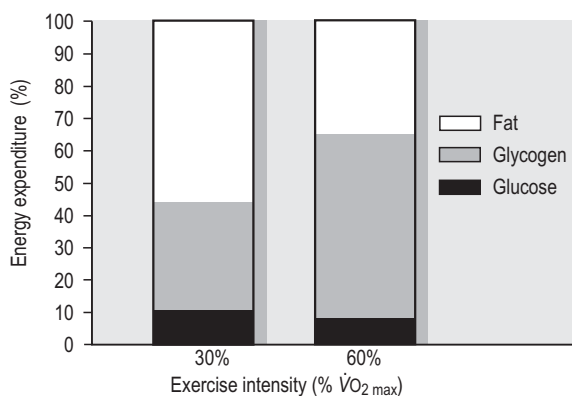
more evident in association with endurance-type exercise,^{79,82,122} but a decrease also may occur after short, near-maximal exercise.¹⁴²

Phosphocreatine (PCr), a high-energy phosphate stored in muscle, is depleted during high-intensity exercise.^{21,109,135,144} The synthesis of PCr after exercise is very rapid and thus results are greatly influenced by the time of sampling in relation to end of exercise. ATP concentration starts to decline and ADP begins to accumulate when ATP consumption exceeds the capacity of ADP rephosphorylation. The myokinase reaction ($\text{ADP} + \text{ADP} \rightarrow \text{ATP} + \text{AMP}$) is activated to keep the concentration of ADP low. To drive this reaction towards formation of ATP, AMP is further deaminated to inosine monophosphate (IMP). The accumulation of IMP closely mirrors the loss of ATP.¹²⁷ This reaction cascade eventually leads to loss of adenine nucleotides and fatigue will follow.

Partitioning of energy supply at different workloads

The relative contribution of different substrates (i.e. carbohydrate, fat, protein) to fuel metabolism during exercise is determined by a number of factors, including the intensity and duration of exercise, muscle composition (i.e. fiber types), training status, diet, and the type of food consumed before exercise (the effects of habitual diet and pre-exercise feeding on metabolic response are further discussed below). At rest when energy transduction is fully aerobic, muscle may use volatile fatty acids, lipids, and carbohydrates. Equine diets are usually low in lipids, and plasma NEFA concentration is also low at rest. Thus, during periods of inactivity, the utilization of lipids for energy may be limited by their low concentration. The main energy sources are acetate, which is formed by bacterial fermentation in the colon and cecum, and glucose.⁸⁵

Contemporary metabolic studies in horses have utilized stable isotope tracer methodology in combination with indirect calorimetry (O_2 uptake measurements and respiratory exchange ratio) for calculation of substrate oxidation rates during exercise at different intensities.^{54–56,58,59} Figure 5.1.8 depicts the estimated relative contributions of lipid, plasma glucose, and muscle glycogen to energy expenditure in horses during exercise at 30% and 60% of $\dot{V}\text{O}_{2\text{max}}$. During the early phases of moderate-intensity exercise (35–55% $\dot{V}\text{O}_{2\text{max}}$), use of carbohydrate (plasma glucose, muscle glycogen) predominates¹⁴⁵ but, if exercise is continued

**Fig. 5.1.8**

The estimated relative contributions of lipid, plasma glucose, and muscle glycogen to energy expenditure in horses during exercise at approximately 30% and 60% of maximum aerobic capacity ($\dot{V}O_{2\max}$). When compared to exercise at 30% $\dot{V}O_{2\max}$, there is increased reliance on muscle glycogen (and a decreased energy contribution from fat) for energy transduction during exercise at 60% $\dot{V}O_{2\max}$. (Adapted from Geor et al.⁵⁶)

for longer periods, there is increased utilization of fats, as indicated by a progressive decrease in the respiratory exchange ratio.^{54,82,122} However, the utilization of plasma glucose and muscle glycogen continues, and is reflected by lowered blood glucose concentrations and depletion of muscle glycogen after endurance exercise.^{67,79,110,111}

The main energy source at maximal performance is muscle glycogen. This is directly demonstrated in studies which show diminished anaerobic power in horses that perform high-intensity exercise after glycogen depletion.¹⁴⁶ Lipids are also used during intense exercise as demonstrated by decreases in NEFA concentrations during intense exercise bouts.^{80,120,121}

Effects of ambient temperature

Exercise causes a decrease in plasma volume²⁸ and if exercise is undertaken in high temperature and humidity, high rates of sweat fluid loss will exacerbate the reduction in plasma volume.³⁵ In extreme conditions, sweating rate may be 10–15 L/h¹⁴⁷ and during the cross-country phase of a three-day event a horse may lose 2–4% of bodyweight, while a 10% loss of bodyweight may be incurred during endurance rides.^{147,148} If the horse is unacclimatized, exercise in hot, humid conditions will decrease maximum oxygen uptake and increase lactate production, i.e.

the primary change being a decrease in aerobic capacity with increased reliance on anaerobic metabolism.¹⁴⁹

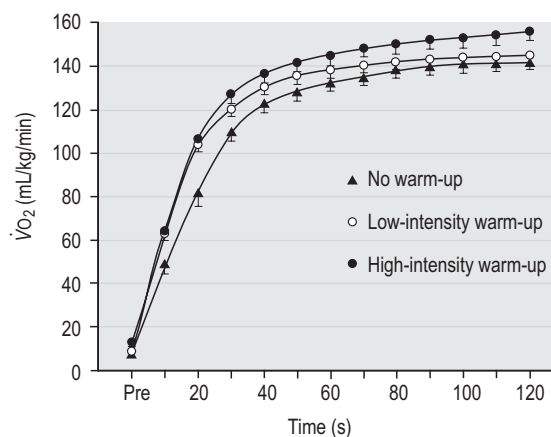
The opposite, exercise in cold, dry conditions (–25°C), does not change indices of aerobic capacity. However, respiratory rate is lower during exercise in cold when compared to thermoneutral ambient conditions.¹⁵⁰ Resting metabolic rate may increase by 70% from the values at thermoneutrality in cold-stressed horses.¹⁵¹

Effects of warm-up

The purpose of a warm-up is to adjust the body to transition from rest to exercise and thereby gain the dual benefits of enhanced performance and reduced risk of injury by a gradual increase in exercise intensity. An active warm-up increases body and muscle temperature by about 1°C as a result of the heat generated during muscular activity. Warm-up improves tissue oxygenation, because epinephrine (adrenaline) and norepinephrine (noradrenaline) released during warm-up increase respiratory rate, tidal volume, and heart rate, cause the spleen to contract and release stored RBC into the circulation, and increase blood flow to skeletal muscles. Catecholamines also activate glycogenolysis and lipolysis and thus increase the supply of energy fuels to the muscle, while elevated temperature decreases the activation energy of metabolic reactions. A higher temperature also increases elasticity of muscles, tendons, and ligaments, which may reduce the risk of injury and allow for full range of motion in the joints.

Two equine studies have demonstrated that a brief period (5–10 min) of warm-up exercise, completed 5 min before the start of treadmill exercise at 115–120% of $\dot{V}O_{2\max}$, results in a significant acceleration in $\dot{V}O_2$ kinetics, i.e. the rate of rise in $\dot{V}O_2$ after exercise onset.^{152,153} This change in oxygen uptake kinetics enhances the ability of muscle to work aerobically and reduces lactate accumulation during high-intensity exercise.^{152–154} In one study, both a low- and a high-intensity warm-up resulted in a significant increase in the run time to fatigue during intense treadmill exercise when compared to exercise undertaken without prior warm-up¹⁵⁴ (Fig. 5.1.9)

The ideal duration and intensity of warm-up exercise is not known and likely depends on the nature of the subsequent exercise task and the prevailing ambient conditions. On the one hand, if the warm-up period is too brief to allow time for the rise in body

**Fig. 5.1.9**

Effects of a low- and high-intensity warm-up on oxygen consumption ($\dot{V}O_2$) in six horses during 2 minutes of intense exercise (~115% of maximum aerobic capacity). Note the acceleration in the rate of rise in $\dot{V}O_2$ when exercise was preceded by a warm-up. (Adapted from Geor et al.¹⁵³)

temperature, the desired effect is not gained, whereas if exercise intensity during warm-up is too high, significant lactate accumulation will be present at the onset of performance exercise. The studies by Tyler et al.¹⁵² and Geor et al.¹⁵³ demonstrated that even a low-intensity warm-up (5 min of trotting exercise) is beneficial in terms of an enhancement in aerobic energy metabolism during subsequent galloping exercise. The benefits of warm-up are likely diminished if the rest period between warm-up and performance is extended so that the horse cools down.

Economy of locomotion

Stride length and stride frequency are the main determinants of running speed. Net energy cost, which is expressed as mL O_2 consumed over distance covered, varies minimally over a great variety of speeds because as speed increases horses change gait (walk, trot, canter, gallop) to an energetically more efficient one. Within a given gait, energy cost changes with increasing speed along a U-shaped curve which overlaps with the respective curve for the adjacent gait.^{155,156} The change in gait occurs at the crossover point where net energy cost is the same for the two gaits, e.g. walk and trot, or trot and gallop. This relationship has been

Table 5.1.2 Maximum oxygen uptake ($\dot{V}O_{2\max}$) in different breeds

Breed	$\dot{V}O_{2\max}$ (mL/kg/min)	References
Thoroughbred	133 ± 10 , 135.8 ± 5.9 ; 154 ± 3	161–163
Standardbred	143.9 ± 10.7 , 151 ± 2 ; 164.9 ± 4.3	164–166
Arab	129 ± 2.5	163
Pony	107.8 ± 12.8	166
Donkey	110 ± 2	133

Values are means $\pm$ standard error of the mean.

described in detail by Hoyt & Taylor (Fig. 5.1.10).¹⁵⁵ When allowed to choose gaits, the horse has a tendency to utilize voluntarily a relatively narrow set of speeds around the nadir of the energy cost curve. Possible explanations for this behavior include minimizing musculoskeletal stresses and maximizing metabolic economy.¹⁵⁷ Locomotion pattern also depends on other factors, the most important of these being muscle composition.^{158,159} These factors may have performance implications, especially in Standardbred events (trotting or pacing) where the horse is forced to maintain a certain gait for an extended period of time and at speeds that are not energetically optimal for the gait in question (Fig. 5.1.10).

Differences between breeds

Thoroughbreds have higher maximum oxygen uptake than other breeds (Table 5.1.2) and thus their aerobic capacity is also higher than in other breeds. Another basis for differences in metabolic responses is muscle fiber composition. In faster breeds, such as Thoroughbreds, the percentage of type I fibers in locomotor muscles is lower than in Standardbreds, which again have lower percentages of type I fibers than horses used for endurance events, such as Arabs.¹⁶⁰ The breeds with a high percentage of type I fibers are more suited for aerobic, long-lasting work while horses with a high percentage of type II fibers are best suited for exercise in which high-intensity bursts are needed. Within a single breed, the variation in the slow-twitch: fast-twitch (type I:type II) ratio is less marked than between breeds, and training-induced differences are mainly seen in the type IIA:type IIB ratio. Exact comparisons are, however, difficult because horses from

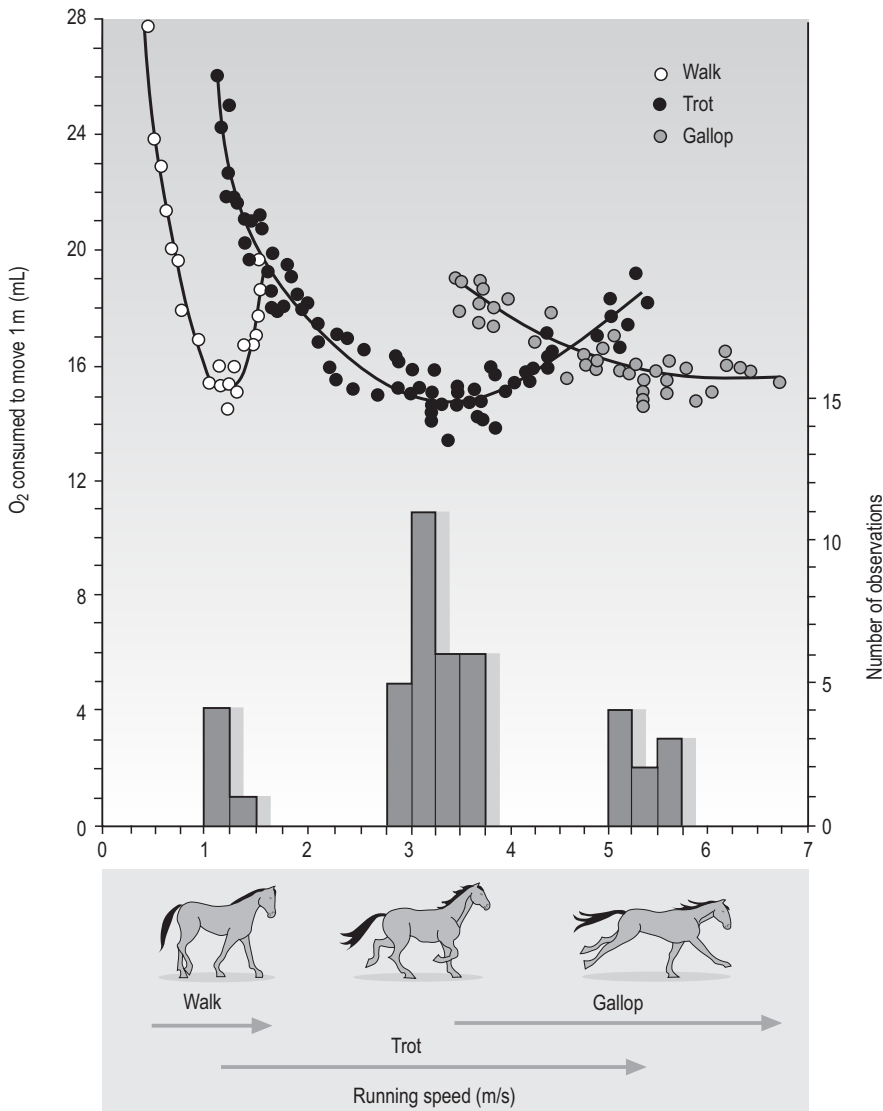


Fig. 5.1.10

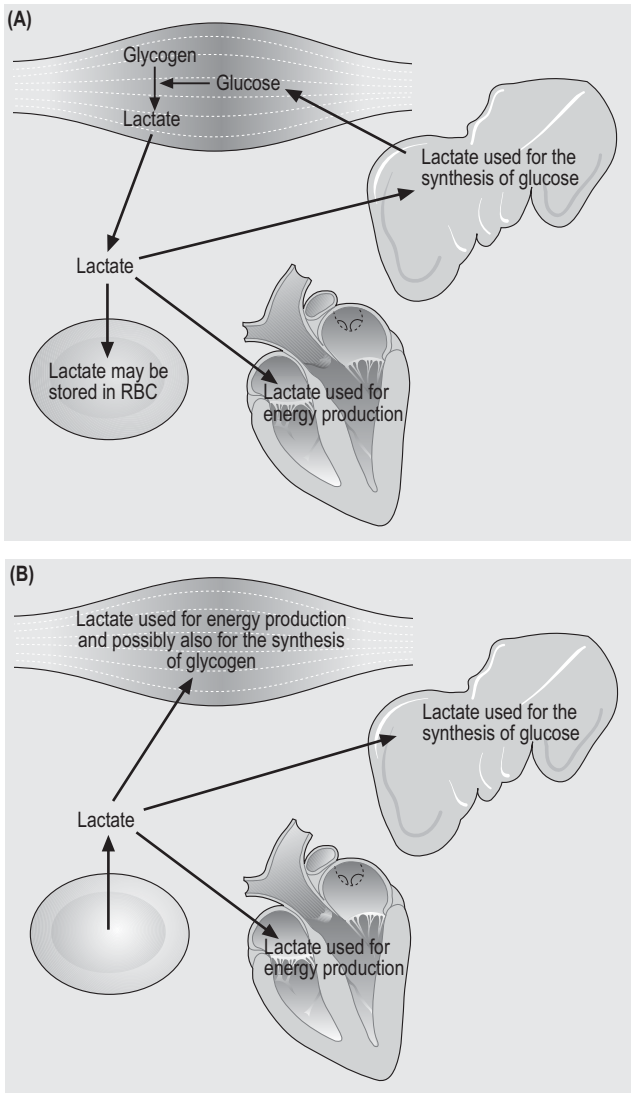
The relationship between net energy cost, expressed as mL O_2 consumed to move 1 m, and speed at the walk, trot, and gallop in small horses. Notice that the minimum energy cost is similar for all gaits. If allowed to do so, the horse changes gait at the speed where energy cost of the two adjacent gaits is the same. (Figure originally published by Hoyt & Taylor¹⁵⁵ and reprinted with the permission of Nature Publishing Group.)

different breeds are used for different purposes and they hardly ever perform comparable exercises. This is evident when maximal lactate concentrations after different events are compared (see Table 5.1.1). For faster breeds, it is possible to compare the metabolic effects of races (e.g. Thoroughbred or Standardbred racing) that may be considered as maximal effort, although differences in gait complicate these comparisons.

Metabolism during recovery from exercise

Lactate removal

After cessation of exercise, the rate of oxygen consumption remains elevated¹⁶⁷ and blood lactate concentration continues to increase, with peak

**Fig. 5.1.11**

During exercise (A) lactate is formed in the working muscles and the flow of lactate is from muscle cells into blood plasma and further into tissues such as heart and liver that may use lactate for energy or glucose production. In horses, a substantial proportion of lactate produced in muscle may also be taken up by red blood cells (RBC). During recovery (B) the direction of lactate flow is reversed, i.e. from RBC to plasma, and the main user of lactate is skeletal muscle. Light exercise during the recovery period that increases muscle energy requirements will also increase the rate of lactate disappearance.

concentrations attained 2–10 min post-exercise. Lactate is an excellent energy source because it contains more than 90% of the energy in glucose. During exercise the main tissues that use lactate are the liver, which converts lactate back to glucose, and the heart, in which lactate is used directly for energy. There also may be some utilization of lactate by type I muscle fibers. However, during recovery, the major consumer of lactate is, due to its large mass, skeletal muscle with oxidation of lactate by all fiber types.¹⁶⁸

The rate of lactate removal from blood depends on the metabolic state. At rest the need for energy is low, but even light exercise increases the oxygen consumption of muscles and thereby the oxidation of lactic acid.^{168–170} Following high-speed treadmill exercise, the half-life of lactate is decreased by 50% in horses

that exercise lightly in comparison to those that remain stationary.¹⁶⁹ The rate of lactate disappearance is independent of concentration, which implies that the rate-limiting step in the process is saturable¹⁶⁹ (Fig. 5.1.11).

Glycogen synthesis

Recovery from exercise is metabolically characterized by resynthesis of energy stores depleted during exercise, especially that of glycogen. In horses, recovery of glycogen is typically slow; during the first 24 hours practically no repletion occurs and full recovery after exhausting exercise may take up to 3 days.^{78,80,110,112} In horses, glycogen resynthesis appears to start in type

IIB fibers,¹¹² and among the two glycogen forms, synthesis of proglycogen is favored during the early phase of recovery in both humans and horses.^{141,171} In human athletes, consumption of a high-carbohydrate diet enhances the rate of glycogen resynthesis, but similar abrupt changes in feed, especially the addition of soluble carbohydrates, are not practical in the horse because they may cause gastrointestinal dysfunction or even laminitis. The only means that has so far been found to enhance the rate of muscle glycogen synthesis is the intravenous administration of large doses of glucose (6 g/kg over 12 hours).^{146,172}

The reasons for the slow rate of glycogen synthesis in the horse are not known, but in general the hormonal status immediately after exercise does not favor anabolic processes, such as the resynthesis of glycogen. During exercise, the concentration of insulin is reduced.^{117,118} As insulin contributes to the activation of glycogen synthase as well as the transport of glucose into muscle by the insulin-activated glucose transporter (GLUT-4), low insulin status during the post-exercise period does not favor glycogen resynthesis. Another possible factor may be a shortage of fuel during recovery. Skeletal muscle has to maintain its normal functions and only the available substrate (glucose) in excess of these demands is available for the resynthesis of glycogen. After exercise, catecholamine concentrations decrease within minutes and consequently lipolysis is inhibited and the plasma concentration of NEFA decreases to a very low level.⁸⁰ Therefore, the predominant substrate available for muscle will be glucose (Fig. 5.1.12).

Metabolic responses to training

Exercise training affects aerobic and anaerobic capacity and thus training effects are seen at both submaximal and maximal exercise intensities. Changes in aerobic capacity reflect increases in maximum oxygen consumption,^{74,162,166,173,174} blood volume, and red cell volume in young horses,⁴ capillarization of all fiber types,¹⁷⁵ and increases in the mitochondrial density and activities of oxidative enzymes such as CS and HAD.^{88,160,176–181} Such increases in aerobic capacity mean that the trained horse is able to run faster before the lactate threshold is reached and accumulation of lactate begins when compared to an untrained animal.¹⁴²

The primary change in muscle fiber composition is from IIB/IIX → IIA and further training also may result in an increase in type I fibers.^{177,180,182–184} Histochemical analysis shows an increase in type IIA fibers, but recent use of myosin-specific antibodies has revealed that after training muscles contain a high proportion of IIXA fibers.¹⁸⁵ The best Standardbred trotters have the highest type IIA:IIB fiber ratios and also the cross-sectional area of type II fibers tends to be smaller when compared to less well-performing horses.¹⁷⁵ In contrast, successful endurance horses have a high percentage of type I fibers with a large cross-sectional area.¹⁸⁶ Very intense training has been reported to increase the cross-sectional area of type II fibers.¹⁸⁷ The most important changes that describe training effects in skeletal muscle are increases in type I fiber area and capillarization, particularly the

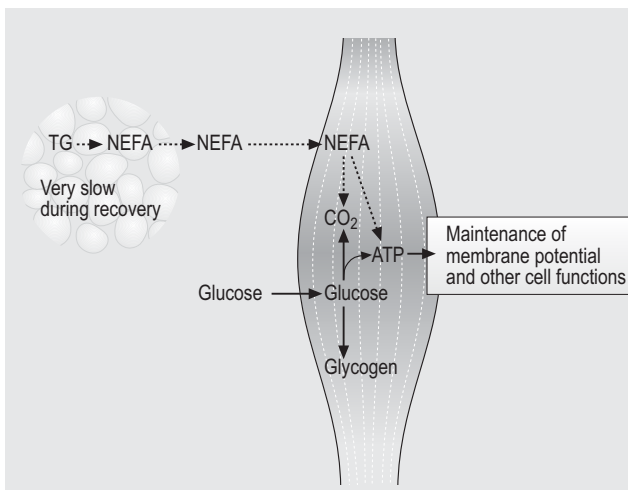


Fig. 5.1.12

During recovery the rate of lipolysis decreases, leading to low concentrations of fatty acids (NEFA) in plasma. Therefore, the predominant substrate for muscle metabolism is blood glucose. The use of glucose in the maintenance of cell functions will affect the availability of glucose for the resynthesis of glycogen. ATP, adenosine triphosphate; TG, triglycerides.

number of capillaries in contact with type I and type IIA fibers, and an increase in the oxidative capacity of type IIB fibers.^{188,189} Some studies have also demonstrated an increase in muscle glycogen and triglyceride content after endurance training.¹⁸⁴

Because of increased oxidative capacity, less glycogen is used and respiratory exchange ratio decreases during both high-¹⁹⁰ and moderate-intensity⁵⁷ exercise. Also due to high aerobic capacity, lactate accumulation in plasma at a given submaximal work level is lower.⁹¹ Studies in humans also demonstrate that the exercise-induced increase in muscle lactate concentration is mitigated by training¹⁰⁷ due to increased lactate efflux out of the muscle and a training-associated increase in plasma volume.

The changes in anaerobic capacity are more difficult to measure, but an increase in maximal accumulated oxygen deficit (MAOD) and lower accumulation of lactate in skeletal muscle were found in trained horses after a maximal sprint to fatigue, providing indication of an increase in anaerobic capacity with training.¹⁶² These changes were accompanied by a significant increase in run time to fatigue during sprinting. The effects of training on blood lactate concentrations after maximal effort are controversial. Some studies show no differences¹⁶² while others show significantly higher lactate concentrations after training.^{91,120} As previously mentioned, studies in human athletes have demonstrated that training increases the quantity of MCTs on muscle membranes.^{105,106} This increase in muscle MCT could contribute to higher blood lactate concentrations after training. Another factor that could modify blood lactate concentrations after training is an increase in muscle buffering capacity. Studies in horses have reported both an increase and no change in muscle buffering capacity with training.^{162,176,191}

Training intensity and duration

Significant training effects are apparent even when the intensity of training exercise is low or moderate, but these changes mainly reflect an increase in aerobic capacity and reactions related to aerobic metabolism. Decreases in the rate of glycogenolysis, increases in oxidative enzymes, and increases in oxygen extraction by tissues are evident after as little as 2 weeks of training.^{173,174} sometimes even before any measurable changes in indices of muscle oxidative capacity can be detected.¹⁹⁰ In general, most of the training-induced increase in aerobic capacity occurs during the initial

6-week period of training.¹⁶⁵ However, further increments in $\dot{V}O_{2\max}$ occur if a training stimulus is maintained.¹⁶⁵ The study by Hinchcliff et al.¹⁶² however, clearly shows that high-intensity training is necessary for improvements in anaerobic capacity. It is possible that intense exercise is also necessary for maximum improvements in aerobic capacity because adaptive changes in aerobic processes appear to require hypoxia as an inducer of gene activity, e.g. the induction of erythropoiesis, capillary growth, and the synthesis of glycolytic enzymes appear to be regulated by hypoxia.¹⁹²

Detraining

Most of the changes in metabolism require synthesis of new proteins and thus require days or weeks to develop; these adaptations also persist for several weeks after the cessation of regular physical activity (detraining).^{193,194} In general, adaptive training responses in skeletal muscle persist for 5–6 weeks after the cessation of regular physical activity.^{180,184} Similarly, there is minimal change in maximum oxygen uptake after 2–4 months of detraining.^{165,194} However, in the study by Butler et al.,¹⁹⁴ lactate accumulation during intense exercise was higher after a 15-week period of detraining, indicating a decrease in aerobic capacity. The fiber type changes during detraining occur in reverse order to the changes during training.^{180,184}

Effects of diet on metabolic responses

The chemical energy (i.e. ATP) required for muscle contraction is ultimately provided in the diet. Therefore, inasmuch as diet can influence the storage of glycogen and TG in muscle, there is an obvious relationship between dietary intake and skeletal muscle exercise metabolism. Indeed, studies in human athletes have unequivocally demonstrated that a high-CHO, low-fat diet will result in higher muscle glycogen concentration when compared to a more conventional, lower-CHO diet.¹⁹⁵ Furthermore, there is a direct relationship between initial muscle glycogen stores and performance during moderate-intensity (50–80% of $\dot{V}O_{2\max}$) endurance exercise in humans.¹⁹⁶ Thus, for human athletes, dietary strategies that boost muscle glycogen stores enhance exercise performance. Conversely, a low-

CHO, high-fat diet impairs endurance exercise performance, in part due to lower initial muscle glycogen concentrations.

Although there has been extensive study of the metabolic responses to exercise in horses, relatively few studies have examined the effects of diet composition on substrate metabolism and exercise performance. Traditionally, the high energy requirements of athletic horses have been met by diets high in hydrolyzable CHO (i.e. grains). In the past decade, however, there has been increased emphasis on use of alternative energy sources, such as fat and beet pulp, in equine rations and some data are available regarding the effects of such diets on the metabolic response to exercise.

High carbohydrate diets

Only a modest increase in the glycogen content of muscle has been observed in horses fed diets rich in hydrolyzable CHO.^{81–83} For example, Essén-Gustavsson et al⁸³ reported a 12% increase in the resting muscle glycogen content of Standardbred horses fed a diet containing approximately 2 kg of starch and sugar when compared to an isocaloric diet that provided about 1.3 kg hydrolyzable CHO per day. However, no beneficial effect on performance has been shown in horses after CHO-rich diets.^{81–83} On the contrary, higher heart rate (HR) and blood lactate accumulation are observed during intense exercise in horses fed CHO-rich diets.^{81,82} The underlying mechanisms of these responses are unknown but may be related to alterations in sympathetic outflow and circulating catecholamine concentrations. Interestingly, Jansson et al¹⁹⁷ reported higher HR during submaximal exercise in horses fed 1.5 kg of barley sugar per day when compared to an oat-based diet, suggesting that the form of CHO also may influence the HR response during exercise. There is also evidence that diets comparatively high in starch and sugar increase HR and excitability of horses at rest.¹⁹⁸

Fat-supplemented diets

The inclusion of fat (as vegetable oil) in diets for athletic horses is now widespread and it is common for horses to receive up to 20% of total daily digestible energy (DE) from fat. However, the effects of dietary fat supplementation in horses on exercise metabolism

and performance are somewhat controversial, in part because of the wide variability in the design and results of studies examining various physiological responses to dietary fat.

Fat supplementation in horses is characterized by an increase in plasma phospholipids and cholesterol and a decrease in plasma TGs.^{199,200} Changes in the activities of lipoprotein lipase and the enzymes of β -oxidation also suggest that horses adapted to a fat-supplemented diet have increased capacity for the uptake and oxidation of fatty acids in muscle. Indeed, some recent studies have shown lower respiratory exchange ratio in fat-adapted horses during low- and moderate-intensity exercise.^{59,201} Pagan et al⁵⁹ also reported a decrease in glucose flux during low-intensity exercise in horses adapted to a diet providing 25% of DE from corn oil. Thus, fat supplementation enhances lipid oxidation and spares the use of endogenous CHO (plasma glucose, muscle glycogen) during moderate exercise. Theoretically, such a glycogen-sparing effect could improve exercise performance. However, the actual effects of fat supplementation on endurance exercise performance have not been reported.

The minimum (or optimum) level of dietary fat necessary for expression of these metabolic adaptations has not been established. Most studies have fed diets containing 3–12% fat (total diet on a dry matter basis) and in one study a dose–response relationship was detected between fat intake (3.0–10.8% fat) and heparin-released plasma lipoprotein lipase activity.²⁰²

The length of time required for metabolic adaptation to dietary fat is a somewhat contentious issue. Some nutritionists believe that a minimum of 10–12 weeks is required for adaptation.²¹⁷ However, metabolic adaptations to fat supplementation have been observed as early as 3–5 weeks after the start of supplementation.^{59,199} Thus, whereas a 2–3-month period may be required for complete adaptation to a fat-supplemented diet, some of the metabolic responses are evident much earlier. In one study, the metabolic responses to fat supplementation were abolished within 5 weeks of withdrawal of the oil-supplemented diet.¹⁹⁹ Thus, the putative benefits of fat-supplemented diets in horses are dependent on continued use of such rations.

There is some evidence that fat supplementation also improves the performance of horses undertaking higher-intensity exercise (e.g. race horses). Harkins et al²⁰³ reported improved racetrack performance in Thoroughbreds fed a fat-supplemented diet and attrib-

uted this improvement to increased muscle glycogen content and glycogen utilization rate during exercise. Furthermore, Eaton et al²⁰⁴ reported that a fat-supplemented diet resulted in a small but statistically significant increase in run time to fatigue and MAOD in horses undertaking treadmill exercise at an intensity equivalent to 120% of $\dot{V}O_{2\text{ max}}$. However, in this study there was no corresponding change in resting muscle glycogen content or glycogen utilization rate during exercise. The mechanism for enhancement of high-intensity exercise performance with fat supplementation is unclear, although increased activation of glycolysis and glycogenolysis is a possibility, the data of Eaton and colleagues²⁰⁴ notwithstanding.

Fat-supplemented diets for horses have lower hydrolyzable CHO (starch and sugar) content when compared to grain-based rations. These diets therefore provide less substrate (glucose) for muscle glycogen synthesis. However, although a drastic reduction in the soluble CHO content of the diet (<10% of DE from starch and sugar) is associated with marked reductions in muscle glycogen content,⁸¹ it appears that muscle glycogen content is largely unchanged when horses are fed diets containing moderate amounts of fat (up to 7.5% of total dry matter or 20% of DE).^{84,198} However, in horses not adapted to a fat-supplemented diet, consumption of meals containing 7.5% fat (rapeseed oil) slows the rate of muscle glycogen replenishment.⁸⁴

Sugar beet pulp

Non-starch carbohydrate feeds, such as sugar beet pulp (SBP) and soya hulls, are now commonly added to diets for athletic horses. The carbohydrate fraction of these feeds is devoid of starch but rich in non-starch polysaccharides (fiber). SBP contains major fractions of pectins, arabinans, and galactans that are extensively fermented in the hindgut.²⁰⁵ Up to 3.0 g SBP per kg bodyweight have been fed to adult horses without any adverse effects on overall nutrient utilization or performance.²⁰⁶ Replacing oats with plain SBP will reduce the glycemic and insulinemic responses to a meal.²⁰⁶ However, when oats were replaced by molassed SBP there was no appreciable change in glycemic response although the post-prandial increase in insulin was mitigated.²⁰⁷

Replacing oats with SBP also mitigates the rate of muscle glycogenolysis and the increases in muscle and plasma lactate in Standardbred trotters performing a treadmill exercise test that simulated a race.²⁰⁷

Similarly, replacing oats with barley sugar resulted in a significant reduction in muscle glycogen utilization during intense exercise.¹⁹⁷ Therefore, a reduction in dietary starch, with replacement by SBP or barley sugar, modifies the muscle glycogenolytic response to high-intensity exercise. The mechanism of this apparent glycogen-sparing effect when oat starch is replaced by barley sugar or SBP is not known. Potentially, an increase in sugar intake results in enhanced use of plasma glucose for energy with a concomitant decrease in energy transduction from muscle glycogen.

Dietary protein

Studies in humans and in dogs have indicated that protein is an unimportant substrate during exercise, although under some circumstances amino acid oxidation may account for up to 5–10% of energy expenditure.¹⁹⁵ The contribution of protein to energy expenditure in horses during exercise is unknown, but it has generally been assumed that carbohydrate and fat oxidation predominate. On the other hand, the effects of dietary protein level on exercise metabolism and performance have received some attention. In one study of racetrack feeding practices, there was an inverse correlation between dietary protein level and race performance; for every 1 kg of crude protein (CP) ingested over recommended levels, there was a 1–3 s increase in race time.²⁰⁸ However, these conclusions are questionable as the statistical analysis of the data was inappropriate. Subsequent laboratory investigations comparing metabolic responses in horses fed moderate- (~10% CP; recommended levels) versus high- (~18% CP) protein diets have yielded equivocal results. Miller et al²⁰⁹ and Pagan et al⁸² reported attenuated blood lactate accumulation during exercise in horses fed a high-protein diet. However, a subsequent study by Miller-Graber and colleagues²¹⁰ did not detect an effect of dietary protein level on lactate accumulation or muscle glycogenolysis during exercise.

More recently, Graham-Thiers and colleagues²¹¹ have evaluated the effects of a restricted protein diet (7.5% CP with added lysine and threonine) on acid-base responses in horses subjected to repeated bouts of high-intensity exercise. When compared to a 14.5% CP diet, the restricted protein diet mitigated exercise-associated acidemia. However, quantitatively, the effects were very small and the physiological significance remains to be determined (for further discussion, see Chapter 6.2).

Effects of pre-exercise feeding

The timing and composition of a meal consumed before exercise can influence metabolic response. Most notably, the hyperglycemia and insulinemia associated with the digestion and absorption of grain meals affect the mix of substrates utilized during a bout of exercise. Insulin is a potent inhibitor of lipolysis and fatty acid oxidation in skeletal muscle and also promotes glucose uptake into muscle via recruitment of the transporter protein GLUT-4 to the sarcolemma. Thus, hyperinsulinemia at exercise onset will suppress NEFA availability and lipid oxidation and increase reliance on carbohydrate stores (including plasma glucose) for energy transduction.

Accordingly, several equine studies have demonstrated that a grain meal (1–3 kg of oats, corn, or a mixture of the two) consumed 3 hours or less before exercise results in hyperglycemia, hyperinsulinemia, and decreased plasma NEFA concentration at the start of exercise, and a subsequent marked decrease in plasma glucose concentration during the initial period of exercise.^{212–215} This decrease in plasma glucose concentration tends to be short-lived such that during prolonged moderate-intensity exercise (e.g. 60 min at 50% of $\dot{V}O_{2\text{ max}}$), the plasma glucose concentration of grain-fed horses is not substantially different from horses fasted before exercise. On the other hand, plasma NEFA remains lower when compared to the fasted state throughout exercise. Jose-Cunilleras and colleagues⁵⁸ utilized isotopic tracer methods and indirect calorimetry to determine the effects of hay (alfalfa cubes; ~3 kg) and starch (cracked corn; 1.7 kg) feeding on glucose flux and substrate oxidation during exercise. The alfalfa cubes were consumed between 2 and 3 hours before exercise, while the cracked corn was ingested 90 min pre-exercise. Feeding corn before exercise resulted in increased utilization of blood-borne glucose and whole-body carbohydrate oxidation when compared to a meal of alfalfa or not feeding (Fig. 5.1.13).

The effects of pre-exercise grain feeding on endurance exercise performance in horses have not been reported. In humans, carbohydrate ingestion during exercise unequivocally improves performance during prolonged (more than 2 hours) moderate-intensity (>50–60% of $\dot{V}O_{2\text{ max}}$) exercise, presumably by maintaining glucose supply in skeletal muscle at a time when glycogen stores are depleted. On the other hand, the performance effects of pre-exercise glucose feedings in human athletes are more equivocal. For horses performing endurance exercise, the acceleration in

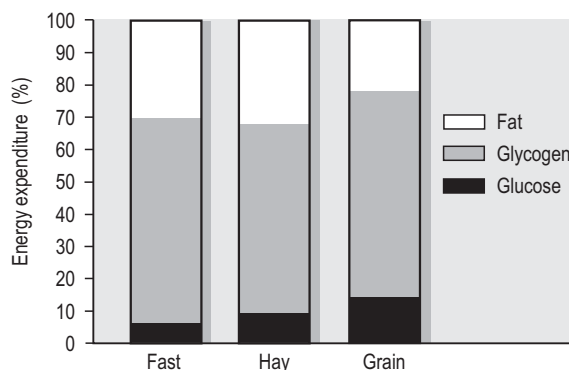


Fig. 5.1.13

Effects of pre-exercise feeding on the partitioning of energy expenditure in horses during the 5–30 min period of exercise at 50% of maximum oxygen uptake. Horses were either fasted or fed cracked corn or alfalfa cubes 2 hours before exercise. Grain feeding resulted in a significant increase in the caloric contribution from oxidation of glucose when compared to the fast and hay feeding trials. (From Jose-Cunilleras et al.⁵⁸)

carbohydrate oxidation (and suppression of fat oxidation) associated with grain feeding may result in premature fatigue as a result of carbohydrate depletion.

As demonstrated in the study by Jose-Cunilleras et al.,⁵⁸ together with the results of earlier studies by Pagan & Harris,²¹⁵ forage meals (<2–3 kg) consumed 2–3 hours before exercise have minimal effect on substrate availability and oxidation during sustained exertion. However, free choice consumption of hay in the 12–24-hour period before exercise may adversely affect performance because of an increase in bodyweight (gut fill). Large meals (hay or grain or a combination) consumed near the start of exercise also may result in a decrease in plasma volume as a result of fluid shifts into the gastrointestinal tract.²¹⁵ Such reductions in plasma volume could compromise cardiovascular and thermoregulatory function during exercise.

There is some evidence that a short-term reduction in forage intake is beneficial in horses undertaking high-intensity exercise. When compared to ad libitum hay consumption, restricting hay intake to ~1% of bodyweight for a 3-day period before a treadmill exercise test (2 min at 115% $\dot{V}O_{2\text{ max}}$) resulted in a 2% decrease in bodyweight and a reduction in anaerobic energy expenditure during exercise, as evidenced by reduced oxygen deficit and plasma lactate concentrations. The reduction in bodyweight was attributed to a reduction in gut fill.²¹⁶

In summary, small forage meals consumed 2–3 hours before exercise have minimal effect on substrate metabolism during exertion. However, meals containing hydrolyzable carbohydrate (starch and sugar) consumed within 3 hours of the start of exercise accelerate carbohydrate utilization and decrease lipid

oxidation. Although the performance effects of these feeding practices are not known, this suppression in lipid oxidation may be detrimental during endurance exercise (e.g. endurance races; speed and endurance test of a three-day event).

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Endocrine alterations in the equine athlete

Kenneth Harrington McKeever & Mary Elizabeth Gordon

Introduction

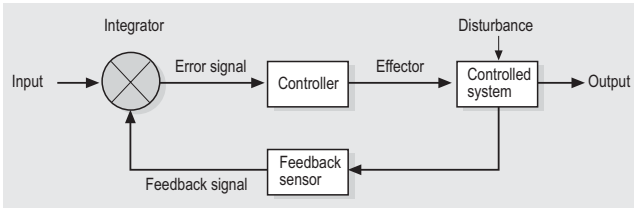
For the most part the domestic horse spends a good part of its day either eating or seeking food.¹ In feral horses the latter can involve treks over a wide range.¹ However, most horses spend the majority of their day eating, standing, and occasionally exercising.¹ Exercise can range from running up and down the fence line in anticipation of the feed truck to athletic training for a variety of competitive endeavors.¹ Under resting conditions, the horse has a relatively easy job of maintaining the internal environment. However, whatever the activity, the performance of work or exercise is a major physiological challenge, a disturbance to homeostasis, that invokes an integrative response from multiple organ systems.¹ On a gross level there is a pairing of those systems associated with convective transport and those associated with the transduction of potential energy into kinetic energy.¹ Put another way, the response to exercise requires the transport of oxygen from the atmosphere to the cells in the working muscles where it is utilized in metabolic pathways generating adenosine triphosphate (ATP) for fuel utilization.¹ In reality, though, the adjustments to acute exercise require the coordination of several systems including the respiratory, cardiovascular, muscular, integumentary, renal, and hepatic systems, and the various organs of the digestive tract.¹⁻⁵ Each tissue or organ called upon to facilitate movement must function in coordination with others in a variety of classic feedback loops (Fig. 5.2.1). Multiple layers of control exist in the body to facilitate work. The first muscle contractions associated with work will alter mechanisms of autoregulation causing changes in local control of the local environment that are sensed peripherally. Longer work causes system-wide alterations that necessitate integrated whole-body responses requiring neural and endocrine mediation.

The most rapid mechanisms used to facilitate a coordinated response to exercise involve an integration of error signals in the periphery that are communicated via the nervous system to central command centers where adjustments are made to the respiratory and cardiovascular systems.³⁻⁵ Some have suggested that the rapid adjustments in cardiopulmonary function at the onset of exercise can be accomplished primarily via a shift in autonomic tone with a withdrawal of parasympathetic tone and an increased sympathetic drive.^{4,5} However, as exercise progresses beyond a few seconds, more sophisticated mechanisms are called upon to fine-tune the initial response to the disturbance of exercise.

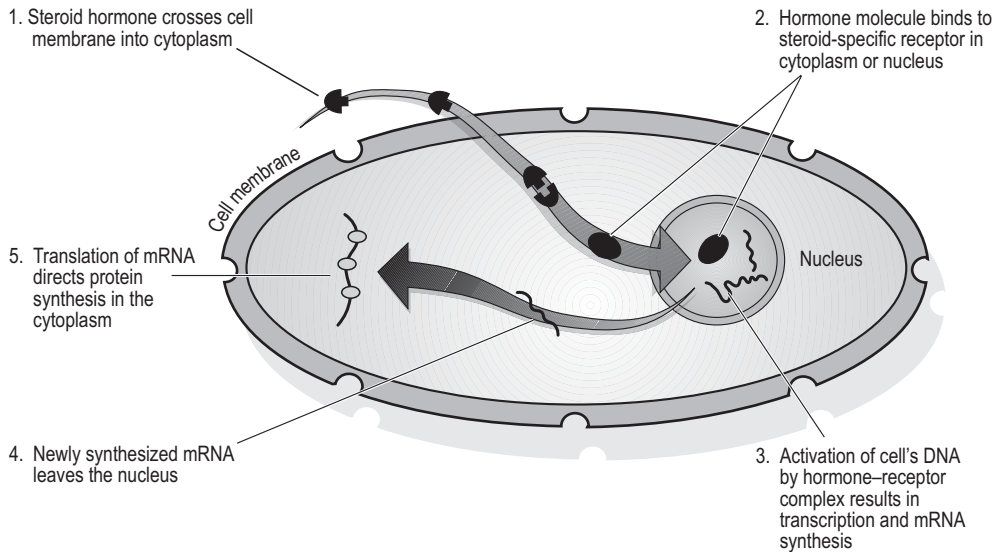
Fine-tuning of the response to exercise that lasts longer than a few seconds is reliant on the regulation of several key variables governing the cardiopulmonary, vascular, and metabolic response to exercise.³⁻⁵ Regulation allows the internal environment to be maintained within relatively narrow limits so as to enhance optimal cell function. This type of classic regulation involves a multiple system response where some variables are controlled so that those most vital to the defense of the internal environment can be regulated. This type of integrative response is slower than a neural response because it requires communication between systems that relies on the secretion of substances by one tissue or organ that are transported remotely to other tissues or organs to evoke a response to the disturbance.³⁻⁵

Endocrine system and hormones

By definition, a hormone is a substance that is produced and released by one organ or tissue and is transported via the blood to a remote target organ or tissue

**Fig. 5.2.1**

Components of a controlled negative feedback system.

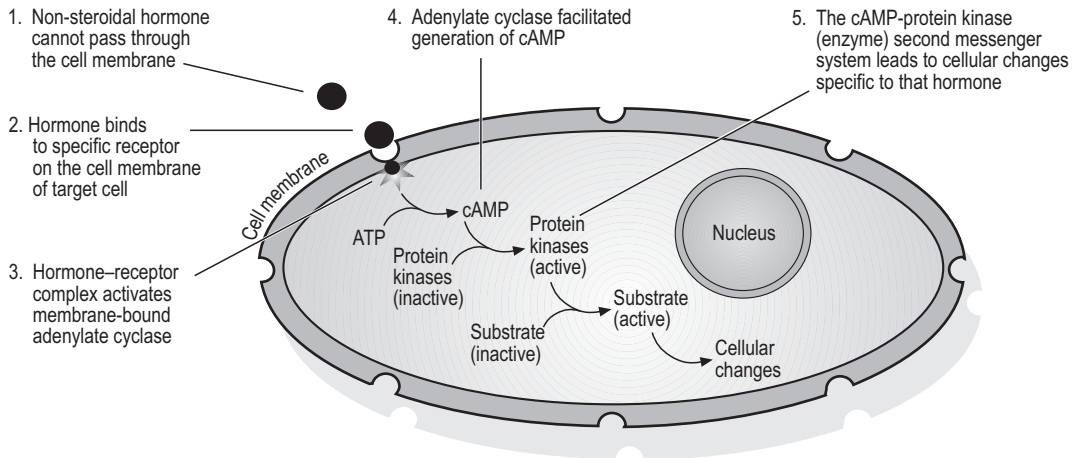
**Fig. 5.2.2**

Gene activation mechanism associated with the action of a steroid hormone.

where it causes a physiological response.^{2,5} Most hormones fall into two major categories: the steroid hormones and the non-steroid hormones. Steroid hormones include cortisol, aldosterone, and the reproductive hormones testosterone, estrogen, and progesterone.^{2,5} The steroid hormones have a classic ring structure and are lipid-soluble, a characteristic that allows them to diffuse across cell membranes.^{2,5} Mechanistically, the steroid hormones exert their effect through direct gene activation that occurs after diffusion into the cell (Fig. 5.2.2).^{2,5} Once across the cell membrane, they bind to receptors in either the cytoplasm or the nucleus.^{2,5} This complex formed by the steroid hormone and receptor induces the DNA in the cell to produce mRNA which, when it enters the cytoplasm, is transcribed, resulting in the production of protein.^{2,5} This protein results in the

physiologic action of the hormone on cellular function.^{2,5}

The non-steroid hormones are not lipophilic and thus cannot pass through the cell membrane.^{2,5} These hormones bind in a lock-and-key fashion to very specific receptors on the surface of the cell membrane (Fig. 5.2.3).^{2,5} The hormone-receptor complex remains in the membrane, but is still able to activate the enzyme adenylate cyclase within the cytoplasm of the cell.^{2,5} Activation starts a cascade that results in the active formation of the enzyme cAMP through the combination of adenylate cyclase and ATP.^{2,5} The enzyme cAMP in turn activates specific inactive protein kinases which cause the conversion of inactive substrates into active substrates that have the capability to induce changes in cellular form or function.^{2,5}

**Fig. 5.2.3**

Second messenger mechanism associated with the action of non-steroid hormones.

Release of most hormones is part of a controlled negative feedback system that keeps a specific variable within narrow limits.^{2,5} The typical negative feedback system is composed of a variable that has an input or set point that is read or sensed by an integrator.^{2,5} For the system to be in a state of balance or homeostasis, the input and output signals must be the same. If the input variable is changed by a disturbance, such as exercise, the integrator senses the mismatch and sends an error signal, usually via nerves, to the controller. The controller then alters the controlled system via an effector. In some cases the effector is a nervous signal; however, in some cases it is a hormone. The effector causes a change in the controlled system, inducing a change in the output that is sensed by the feedback sensor. The feedback sensor sends a signal to the integrator that then determines if the input matches the output of the system.

Physiological variables that are the most critical to normal function are regulated. *Regulation* refers to a phenomenon in which the output of a system remains relatively constant under many different disturbances. For example, mean arterial pressure (MAP), plasma osmolality, blood pH, PCO_2 and PO_2 , plasma electrolyte (Na^+ , K^+ , and Cl^-) concentrations, blood glucose concentration, and body temperature are just a few of the major variables that are regulated and held within very narrow limits during exercise. Regulation of each of these variables is accomplished through a combination of neural and endocrine mechanisms affecting

multiple control strategies. *Control* is a phenomenon where the output of the system constantly changes in order to manage a rate of functioning. In short, some physiological variables are controlled so that others may be regulated. The more rapid responses used to maintain homeostasis are usually mediated by neural control mechanisms with less rapid responses usually mediated by endocrine mechanisms.

For example, maintenance of systemic MAP around a narrow set point is critical to cardiovascular performance.⁴ Multiple systems are controlled to insure that MAP is sufficient to allow perfusion of all the working muscles as well as obligate tissues during exercise.⁴ At the onset of exercise, there is a decrease in total peripheral vascular resistance (TPR) that results from the opening of blood vessels in the working muscles.⁴ This allows for the increase in blood flow to those vascular beds.⁴ However, there is an almost simultaneous increase in cardiac output which comes about through the matching of the input and output signals sensed by volume and baroreceptors placed at strategic points in the cardiovascular system.^{1,4} Matching of the input and output signals (see Fig. 5.2.1) by the vasomotor center of the medulla (the integrator) results in an error signal via the autonomic nervous system (the controller).^{1,4} A withdrawal of vagal tone and an increase in sympathetic drive results in the local release of norepinephrine (noradrenaline, effector), which causes a rapid increase in heart rate and the force of contraction that raises cardiac output (the

controlled system) enough to maintain MAP.^{1,4} As exercise intensity increases, the set point for MAP is increased; thus, higher-intensity exercise usually requires more dramatic responses, including a decrease in blood flow to non-obligate tissues such as the splanchnic vascular beds.^{1,4} The initial part of this response is mediated by neural mechanisms affecting the arteriole in those vascular beds.^{1,4} However, higher-intensity exercise also requires the added influence of endocrine effectors such as vasopressin and angiotensin II to cause sufficient vasoconstriction in non-obligate tissues to facilitate the rise in MAP needed for optimal cardiovascular function.^{1,4} As such, one example of the role of the endocrine system during exercise can be seen in the regulation of MAP.^{1,4} Longer-term control of MAP can be affected by the duration of exercise which results in multiple strategies to control blood volume and defend cardiac filling pressure, cardiac output, and MAP.^{1,4}

Major endocrine glands and hormones

Pituitary gland

The pituitary is found at the base of the brain and is divided into three lobes: the anterior, intermediate, and posterior.^{2,5} Hormones of importance during exercise are produced and released by the anterior and posterior lobes.^{2,5} The pituitary is vitally linked to the hypothalamus, an area of the brain with many very specific neural tracts that act as feedback sensors.^{2,5} The hypothalamus also acts as the integrator in the controlled system, exerting control over the pituitary through neural and endocrine mechanisms.^{2,5} The latter include various releasing hormones and inhibitory hormones and other substances.^{2,5} The central location of this hypothalamic–pituitary axis makes it ideal for mediating the control of a wide variety of functions.^{2,5}

Anterior pituitary hormones

Hormones produced by the anterior lobe of the pituitary include somatotrophin (growth hormone, GH, ST), thyrotrophin (thyroid-stimulating hormone, TSH), adrenocorticotrophin (ACTH), endorphins (EN), enkephalins, dynorphins, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin.^{2,5} The

first three play an important role in growth and development in the young animal and metabolism in the adult animal. The endorphins, enkephalins and dynorphins are opiate-like peptide hormones that modulate pain reception and interact with the neural pathways of the hypothalamic–pituitary axis to influence releasing and inhibiting hormones.^{6–8} FSH, LH, and prolactin are considered hormones essential for normal reproduction and lactation.^{2,5} New research in species other than horses has demonstrated that severe or prolonged exertion can alter their release and thus normal reproductive cycles.^{2,5} Furthermore, prolactin appears to play an important role in the response to severe stress, interacting with many of the metabolic hormones.^{2,5}

Somatotrophin (ST)

Somatotrophin affects all the cells in the body, stimulating development and growth in younger animals. In mature animals it plays a vital role in muscle metabolism through its effects on protein synthesis and its role in fat and carbohydrate utilization.^{2,5} The importance of somatotrophin in maintenance of normal physiologic function and its possible role in slowing or possibly even reversing the effects of aging can be seen in some younger adult humans, where somatotrophin ‘deficiency’ results in changes in appearance, decreased lean body mass, decreased immune function, and other ‘sequelae’ of aging.^{9,10} Amazingly, treatment of these individuals with recombinant human somatotrophin results in increased lean body mass, decreased body fat, and increased muscle mass.^{9,10} Chronic somatotrophin administration appears to increase strength and the ability to perform a battery of weight-lifting exercises in aged male humans.^{9,10} While there have been no reported effects of ST on aerobic capacity, the increase in muscle mass and strength has been shown to benefit the quality of life in humans by increasing the ability to do daily tasks such as maintaining balance while walking and climbing stairs.^{9,10} Those human experiments served as part of the justification for several recent studies of the efficacy of recombinant somatotrophin treatment in preventing or retarding functional decline in geriatric humans^{9,10} and in geriatric horses.^{11–14}

Researchers conducting equine studies^{11–15} have asked ‘quality of life’ questions similar to those posed in experiments using aged humans.^{9,10} While those studies demonstrated that equine ST (eST) therapy increases nitrogen retention and improves appear-

ance in geriatric horses, they did not demonstrate any effect of chronic eST administration on bodyweight or the dimensions of several key muscles measured using ultrasonography.¹³ Functionally, chronic eST administration did not alter aerobic capacity or several commonly used indices of exercise performance, at least not in unfit aged mares.¹⁴ Furthermore, data from unfit geriatric horses indicated that eST did not alter lactate tolerance or cause an increase in maximal power that one would associate with an increase in muscular strength, a logical observation since there was no evidence of an increase in muscle mass.¹⁴ Interestingly, studies of geriatric male humans have shown that recombinant somatotrophin therapy results in increased muscle mass and, in some cases, increased strength as measured in standardized tests performed with weight-lifting equipment.^{9,10} However, while ST therapy does appear to increase strength in humans, there are no data to show that ST therapy alters maximal aerobic capacity or enhances endurance performance.^{9,10}

More recent studies of younger horses have demonstrated that administration of eST prevents some of the bone demineralization that occurs in the first months of intense training.¹⁶ Other studies found that there was minimal or no therapeutic benefit of administering eST to prevent tendon or cartilage injuries or to promote wound healing.^{17–19} Another report demonstrated that eST does not alter aerobic capacity or markers of performance in young (~2 years) Thoroughbreds.^{17,18} However, as with the studies of older horses, the experiments performed on younger animals had no way to evaluate the effects of eST administration on muscular strength; thus, data are needed to determine if eST alters strength and power.

Thyrotrophin (TSH)

Release of TSH is stimulated by thyroid-stimulating hormone releasing hormone (TRH) which is produced in the hypothalamus.^{2,5} Studies of humans and other species have demonstrated that acute exercise elicits mixed effects on TSH release.²⁰ The release of TSH appears to be linked to exercise intensity, as mild and moderate exertion does not appear to have an effect on TSH release. However, there appears to be a threshold for stimulating TSH release, as plasma concentrations of this hormone increase only when exercise intensity exceeds 50% of $\dot{V}O_{2\max}$ in humans.²⁰ This breakpoint is common to several hormonal systems including the observed increases in the catechol-

amines, ACTH, cortisol, plasma renin activity (PRA), etc., which may indicate that there is an interplay in the metabolic response to higher-intensity exercise.^{20–24} Interestingly, exercise duration beyond 40 minutes appears to cause an increase in TSH.²⁰ This observed increase during longer steady-state exertion is similar to the response of other metabolic hormones and may be related to substrate mobilization and attempts to prevent the onset of central fatigue mechanisms. In humans, repeated daily exercise also causes the release of TSH, suggesting the ratcheting up of metabolic function with exercise training.^{5,20} Data on the effects of acute exercise and training on TSH in equine athletes are lacking.

Adrenocorticotrophin (ACTH)

As with TSH, the release of ACTH is stimulated by corticotrophin-releasing hormone, which is secreted by the hypothalamus.^{2,5} A number of published papers have demonstrated that exercise causes an increase in ACTH in the horse; however, most of these only report post-exercise values.^{20,25–31} The major conclusion of most exercise studies has been that both high-intensity and endurance exercise cause an increase in ACTH and subsequent increase in cortisol.²⁰ Some researchers have attempted to characterize this as a stress response; however, if one looks at the role of cortisol in substrate mobilization and metabolic control during exertion, then one can see that the ACTH/cortisol response, if in the normal range, is an appropriate response to exertion.^{20,25–32}

A more recent study demonstrated that ACTH increases in a curvilinear fashion with exercise intensity during controlled incremental exercise performed on a treadmill.²⁴ The ACTH response appeared to be highly correlated to the catecholamine and lactate responses to incremental exercise, all of which also increased in a curvilinear fashion.²⁴ When horses were exercised at steady-state speeds (80% or 110% $\dot{V}O_{2\max}$), the ACTH response was rapid, with concentrations increasing in a linear fashion until the end of the exercise test.²⁴ Post-exercise concentrations fell rapidly, returning to baseline within 60–120 min.²⁴ These responses are similar to those reported for humans and other species.^{5,24}

The endorphins, enkephalins, and dynorphins

These peptides are released from the pituitary in response to pain or pleasure.^{6–8} While some classify these substances as hormones, others classify them as

neurotransmitters.^{6–8} Nevertheless, these substances are naturally occurring opiate-like pain suppressors that may allow a horse to tolerate higher-intensity exercise.^{6–8,31–34} The endorphins, enkephalins, and dynorphins play a role in the response to physiological and psychological stress and appear to modulate pain perception.^{6–8} As such, these hormones are markers of stress. However, since some sports medicine specialists have suggested that an overload of duration, intensity, or resistance is needed for exercise training to elicit an adaptive response, then under controlled conditions, the endorphins may play an important role in allowing a horse to tolerate the progressive increases in exercise intensities or longer durations needed to invoke a training response. Mehl and co-workers^{6–8} have demonstrated that a threshold exists for evoking an increase in plasma β -endorphin concentration. This threshold appears to correspond to ~60% of the speed eliciting $\dot{V}O_{2\max}$. Interestingly, this is the same point at which one sees a curvilinear increase in the catecholamines, plasma renin activity, plasma lactate concentration, and several other variables. This suggests a close interplay between these factors in the transition from low-intensity, primarily aerobic exercise to higher-intensity exercise with an increasing anaerobic component.^{21–24,35} Duration of exercise also appears to affect the magnitude of the endorphin response, with greater plasma concentrations as horses approach fatigue.^{6–8} Training appears to alter the endorphin response to acute exertion with greater concentrations observed in the post-exertion peak occurring between 5 and 10 minutes post-exertion.³⁶

Posterior pituitary hormones

Hormones released from the posterior lobe of the pituitary include arginine vasopressin (AVP) and oxytocin. These two protein hormones are actually produced in the hypothalamus by specialized bundles of nerves.^{2,5} AVP is synthesized in cells of supraoptic and paraventricular nuclei and stored in vesicles in the nerve endings located in the posterior pituitary. Vasopressin plays a role in blood pressure regulation and fluid and electrolyte balance. It is for this latter role that some refer to AVP as antidiuretic hormone or ADH.^{2,5} AVP plays a major role in the short-term and long-term control of cardiovascular function during and following exercise.^{2,5} Oxytocin causes smooth muscle contraction in the epididymis of males and the uterus of females and also acts on mammary tissue, causing milk letdown in lactating mares.^{2,5} Oxytocin

does not appear to play a role in the response to exercise.

Arginine vasopressin (AVP)

AVP is a posterior pituitary hormone associated with the acute and chronic defense of blood pressure, plasma volume, and fluid and electrolyte balance.^{1–3,37–39,40} The primary physiological actions of AVP include vasoconstriction and decreased free water clearance.^{3,37–39,40} The mechanism for the release of AVP involves osmoreceptors in the supraoptic and paraventricular nuclei of the hypothalamus and cardiopulmonary baroreceptors in the atria of the heart.^{2,3,37–40} Data from rats and other species have shown that a very small 1–2% decrease in cell volume in the hypothalamus or change in extracellular osmolality of 2–4 mOsm/kg will stimulate AVP secretion and drinking.^{37–39} Exercise causes an increase in plasma AVP that is correlated with both duration and intensity.^{21,35,37–39,41} Comparative data show that AVP is secreted during exercise in concentrations well above the threshold level associated with its antidiuretic effects, suggesting that its extrarenal actions are more important during acute exercise.^{3,37–39,42–45} Extrarenal actions include AVP's action as a powerful vasoconstrictor and an important component in the control of blood pressure during exercise and its action on splenic blood vessels to prevent resequestration of the splenic reserve in the horse.^{3,25,46} Interestingly, some studies suggest that drinking water, especially cold hypotonic water, during exercise may suppress AVP and thirst, leading to hypohydration.^{38–40,47–49} However, sustained elevations in AVP stimulate thirst and drinking after exercise, cause a decrease in free water clearance by the kidneys, and may influence the uptake of sodium and water from the colon.^{3,38–40,49}

In exercising horses, plasma AVP concentration was recently reported to have increased from ~4.0 pg/mL at rest to ~95 pg/mL at a speed of 10 m/s.⁴⁵ It was also reported that the relation between AVP concentration and exercise intensity was curvilinear and did not plateau at speeds producing maximal heart rate.⁴⁵ Another recent paper reported that AVP increases during steady-state submaximal exercise in horses without a change in free water clearance.⁴² However, the increase does not become significant until between 20 and 40 min of exertion.⁴² Two possible explanations were given for the delay in AVP secretion in submaximally exercised horses:⁴² first, a suppression of AVP secretion due to the volume overload sensed by neural pathways associated with the atrial

baroreceptors and the hypothalamus;^{3,42} second, inhibition of AVP release by the increase in atrial natriuretic peptide (ANP) concentrations at the beginning of exercise.^{3,44} Nevertheless, an increase in AVP concentration was seen with prolonged exercise that appears to be related to sweat losses and decreases in body water that altered plasma osmolality and blood pressure.^{3,44,45} Studies of humans have demonstrated that training alters the slope of the AVP response to acute exercise, suggesting a change in the sensitivity to the exercise challenge.^{21,22,35,38,39,41,47,49} No studies have been published on the effect of training on the AVP response to acute exertion in the horse.

Thyroid

The thyroid is located in the neck in close proximity to the larynx region.^{2,5} It plays a major role in the control of basal metabolic rate which has led some to refer to it as the body's 'thermostat'.^{2,5} The two iodine-containing hormones produced by the thyroid, tri-iodothyronine (T3) and thyroxine (T4), act upon all cells in the body, affecting metabolic rate and subsequently energy metabolism.^{2,5,20} The cells of the thyroid have three major actions when it comes to synthesis and secretion of T3 and T4: collection and transport of iodine, synthesis and secretion of the glycoprotein thyroglobulin into the intracellular colloid, and removal of T3 and T4 from thyroglobulin and secretion into the bloodstream.^{2,5,20} The thyroid hormones circulate in the plasma in both a free and a protein-bound form, with protein-bound accounting for 99.98% of the circulating hormone and the unbound form being the active form able to act on cellular metabolism.^{2,5,20} The thyroid also produces calcitonin, an important hormone in the control of calcium metabolism with potent effects on bone mineral density.^{2,5,20}

Tri-iodothyronine and thyroxine

The release of these hormones is stimulated by TSH, which, as previously mentioned, is released during exercise.^{2,5,20} As with TSH, release of T3 and T4 is associated with both the intensity and duration of exercise in humans and horses.^{5,20,50} Irvine⁵¹ demonstrated that training increases the secretion rate of both T3 and T4 by approximately 65%. This would indirectly support the suggestion that TSH, which increases with training in humans and other species, is also increased with training in the horse. Training

also increases the turnover rate of the thyroid hormones.^{5,20}

Calcitonin

In addition to its control of metabolic rate, the thyroid is vital to calcium homeostasis.^{2,5,20} For this function the thyroid synthesizes and produces calcitonin which plays a role in calcium homeostasis by either inhibiting osteoclast activity in bone or, through its action on the kidney tubules, causing an increase in calcium loss by actively inhibiting tubular reabsorption.^{2,5,20} New bone is formed by osteoblasts and reabsorbed by osteoclasts. In young growing horses, both osteoclasts and osteoblasts are active; however, the activity of osteoblasts outpaces that of osteoclasts, allowing for bone growth and development.^{2,20} To this end calcitonin appears to be more important in the young growing animal through its inhibitory action on the osteoclasts. Calcitonin is also important in the healing of fractures.² Chiba and co-workers⁵² documented substantially elevated plasma calcitonin concentrations in race horses with various fractures. Studies have also demonstrated that there is a period of bone demineralization in young Thoroughbreds in the first few months of training.¹⁶ Growing and adult humans who exercise regularly have increased bone density.⁵ While a great deal of work has examined markers of bone turnover, more data are needed to determine whether acute and chronic exertion affect plasma calcitonin concentrations.^{9,52-56}

Parathyroid glands

The parathyroid glands, which are located in close proximity to the thyroid gland, regulate calcium homeostasis by synthesizing and secreting parathyroid hormone or parathormone (PTH) in response to a change in plasma calcium (Ca^{2+}) concentration.^{2,5,20} The hormone PTH has receptors in the intestinal tract, in the osteoclasts in bones, and in the tubules of the kidneys.^{2,5,20} The action of PTH to stimulate osteoclast activity is antagonistic to calcitonin's inhibitory action. The resultant effect is a net bone reabsorption and the release of calcium and phosphate into the bloodstream.² Actions on the bone are relatively slower compared to PTH ability to alter both the uptake and excretion sides of the homeostatic balance equation by acting on the intestine and the kidney tubule.² Parathyroid hormone has a profound ability to enhance the enzymatic pathway that mediates increases in intestinal absorption of calcium and phos-

phate.^{2,5,20} At the same time PTH can act on the kidney tubules where it enhances calcium reabsorption and phosphate excretion.^{2,5} The increase in phosphate excretion counters the increased amount absorbed concurrent with the increase in calcium absorbed by the intestines.^{2,5}

As with calcitonin, most equine research has focused on effects of repeated exercise on markers of bone turnover.^{9,52–56} It is well recognized that nutritional influences can alter calcium and phosphate balance and bone metabolism. Thus, more work is needed to determine whether exercise intensity and/or duration are factors affecting PTH concentrations. Furthermore, data are needed to determine whether exercise alters the synthesis and secretion rate, receptor numbers, and sensitivity and general interplay of PTH and calcitonin in bone metabolism.

Adrenals

The adrenal glands are multilayered organs that sit atop the kidneys.^{2,5} Functionally, the primary layers are the adrenal medulla and the adrenal cortex.² The medullary portion of the adrenal produces epinephrine (adrenaline) and norepinephrine (noradrenaline), which have the potential to affect most cells in the body.^{2,5,20} In general, epinephrine potentiates the response to exercise, causing profound effects on central cardiovascular and respiratory function.^{5,20} It can cause increases in muscle blood flow and can mobilize glycogen and free fatty acids to fuel exertion.^{2,5,20} The adrenal cortex contains three specialized zones: the zona glomerulosa, zona fasciculata, and zona reticularis.² The cortex produces a multitude of steroid hormones that fall into three major categories: the mineralocorticoids (aldosterone), the glucocorticoids (cortisol), and the gonadocorticoids (androgens and estrogens).^{2,5,20}

Hormones produced by the adrenal medulla (the catecholamines)

The release of the catecholamines has its origin in the fight-or-flight response.⁵⁷ This 'stress' response involves the local release of norepinephrine from the sympathetic nerve endings and a systemic release of epinephrine and norepinephrine from the adrenal medulla.⁵⁷ Receptors for the catecholamines are specialized and are divided into two primary categories, referred to as α - and β -adrenergic receptors.⁵⁷ These

two major categories are divided into subcategories, namely α_1 and α_2 and β_1 and β_2 receptors.⁵⁷

Sympathetic nervous activity increases with intensity and duration of exercise; however, measurable changes in plasma catecholamine concentrations are not apparent below 50–70% of maximal aerobic capacity.⁵⁷ Recent papers report that plasma catecholamine levels increase in a curvilinear fashion with increasing exercise intensity and are highly correlated with plasma lactate concentrations.^{23,24,50} The measurable increase appears to coincide with the intensity where one would expect complete parasympathetic withdrawal.^{19,20,22–24} These increases in the catecholamines enhance the increase in heart rate, force of cardiac contraction, and thus cardiac output.^{3,57} The catecholamines also play a role in inducing splenic contraction and the delivery of 6–12 liters of blood into the central circulation at the onset of exercise.^{57,58} Even with this mobilization of reserve blood volume, the demands of exercise may exceed central cardiovascular capacity in the horse; thus, during high-intensity or long-duration exercise, the catecholamines contribute to the vasoconstriction that decreases blood flow to non-obligate tissues.^{3,4,57}

The catecholamines are also vitally integrated into the respiratory response to exercise.^{3,23,57,59,60} At the onset of exercise the β_2 -adrenergic receptor action relaxes tracheal and bronchial smooth muscle, increasing airway diameter and decreasing airway resistance, and thus facilitating movement of greater amounts of air into and out of the lungs.⁵⁷ While the sympathetic system is not directly responsible for the control of ventilation during exercise, the increase in ventilatory drive associated with activation of the motor cortex can be enhanced during exercise by catecholamine secretion and augmentation of the sensitivity of chemoreceptors in the carotid bodies.⁵⁷ During high-intensity exercise ventilation may be further affected by catecholamine release; however, this is more a stress response than a 'normal' response to exercise.⁵⁷

The catecholamines also have major effects on metabolic pathways associated with substrate utilization during exercise.^{5,20,57} Increases in sympathetic activity, more specifically catecholamine concentrations, result in an increase in hormone-sensitive lipase and subsequently an increase in circulating free fatty acids.^{5,20,57} Exercise-induced increases in the catecholamines also cause an increase in glycogen breakdown, resulting in elevations in blood glucose concentrations.^{5,20,57} It has been suggested that one way in which warm-up exercises benefit the athlete is through

activation of these metabolic pathways, allowing for elevated blood concentrations of glucose and free fatty acids prior to race-induced increases in utilization, thus facilitating delivery of substrate to tissues without a significant lag time.^{5,20,57}

The above responses have been well documented during acute exercise; however, recent data suggest that exercise training alters adrenergic receptor numbers and sensitivity in selected tissues.^{5,20,57} For example, β -adrenergic receptor numbers are unchanged in cardiac muscle with training, while α -adrenergic and muscarinic receptor numbers are reduced.^{5,20,57} Both β -adrenergic receptor numbers and sensitivity are increased in skeletal muscle and in vascular and bronchial smooth muscle.^{5,20,57} Changes in receptor number and sensitivity with training may be important with respect to adjustment of drug doses for the animal that has been trained extensively as opposed to an animal that is at the beginning of a training program or one that is being reconditioned following removal from training.^{5,20,57}

Primary hormones produced by the adrenal cortex

Some sources suggest that more than 30 structurally distinct steroid hormones are secreted by the adrenal cortex.^{2,5,20} Of those, the mineralocorticoid aldosterone and the glucocorticoid cortisol are the most important to the physiologic response to exercise.^{2,5,20}

Aldosterone (ALDO)

Aldosterone plays an important role in electrolyte homeostasis, in particular sodium and potassium balance.^{2,3,5,20,61} It is well recognized that ALDO acts on the kidneys to enhance sodium (and chloride) reabsorption and potassium excretion.^{3,5,20,61} It also acts on the intestines to facilitate the uptake of electrolytes and water.^{3,5,20,61} Aldosterone release can be stimulated by decreases in plasma Na^+ or by increases in plasma H^+ , plasma K^+ , plasma ACTH, and/or increased PRA.^{3,38,39,61} However, the most potent of these stimuli is an increase in plasma K^+ .^{3,61} Studies of horses have attempted to identify factors that may stimulate the release of ALDO during exercise.^{14,42,45,61–65} In one study plasma Na^+ was not significantly affected by exercise and thus a decrease in plasma Na^+ did not appear to have been the primary stimulus for ALDO release.⁴² The early mechanism for the release of ALDO appeared to have been a proportional increase in the plasma renin–angiotensin–aldosterone cascade where

an increase in PRA results in the generation of angiotensin I and angiotensin II, with angiotensin II stimulating the production and release of aldosterone.^{38–40,66–68}

The relationship between plasma aldosterone concentration and exercise intensity has been reported for horses running on a treadmill.⁴⁵ Aldosterone increased from concentrations around 20–50 pg/mL at rest to almost 200 pg/mL at a speed of 10 m/s.^{45,69} A linear relation was found between exercise intensity and aldosterone concentration; however, unlike PRA, ALDO concentration did not reach a plateau at HR_{max} .⁴⁵ Another study found that during submaximal exercise, increases in plasma ALDO concentration paralleled changes in PRA; however, the magnitude of the increase in PRA (66%) was less than the relative increase (709%) in plasma ALDO concentration.⁴² The authors concluded that factors other than PRA affected the release of ALDO in the horse.⁴² Of all the parameters reported, a highly significant increase in plasma K^+ concentration may have served as the strongest stimulus for the release of ALDO.³ An increase in plasma K^+ as small as 0.3 mEq/L can be sufficient to stimulate the secretion of ALDO, independent of the renin–angiotensin cascade, through the conversion of cholesterol to pregnenolone or at a later step in the biosynthetic pathway.³ This is consistent with the acute homeostatic requirements of the horse since a major perturbation in electrolyte homeostasis observed during endurance exertion was an increase in plasma K^+ and not a drop in plasma Na^+ .⁴⁵ As with humans, ALDO has a minimal role in the acute response to exercise in horses; however, ALDO concentration remains elevated for hours after exercise and may affect the long-term reabsorption of sodium and water^{40,70,71} by the kidneys and by the intestinal tract.^{69,72}

Cortisol

The major glucocorticoid secreted by the adrenal glands is cortisol; however, some cortisone, corticosterone, and deoxycorticosterone are also produced and found in the plasma.^{2,5,20} Thornton reported that deoxycorticosterone concentrations are very low in the horse.²⁰ Cortisol, cortisone, and corticosterone can be found in a ratio of 16 : 8 : 0.5 in the plasma; thus, most exercise studies have focused on cortisol.^{2,20} Cortisol undergoes diurnal variation with peak levels found in the early morning between 0600 and 1000 and lowest levels found in the late evening and night.^{2,5,20} Some have characterized the glucocorticoids as ‘stress’ hormones.^{2,5,20} However, they are

released under a multitude of normal situations not characterized as stress.² Thus, it is the appropriateness of their release and the magnitude of their release that would indicate whether a given physiologic disturbance (or stressor) can be classified as a mere perturbation or a dangerous stress.

Release of cortisol allows an individual to tolerate and adapt to challenges to homeostasis that occur in everyday life.^{5,20} To this end, the functional effects of cortisol fall into two major categories: substrate mobilization and immune modulation.^{2,5,20} One such challenge is exercise where cortisol stimulates substrate mobilization by enhancing gluconeogenesis and the mobilization of free fatty acids.^{5,20} At the same time cortisol release will decrease glucose utilization by some tissues, sparing it for use by the central nervous system.^{5,20} One could speculate that such an action could delay the onset of central fatigue that occurs during endurance exertion when blood glucose concentrations drop.^{71,73} Cortisol also causes an increase in protein catabolism with a resultant increase in the release of amino acids.^{2,5,20} These building blocks of protein are thus available during exercise as a source of energy when glucose levels begin to drop.^{2,5,20} They are available after exercise to repair tissues and for the synthesis of enzymes involved in many cellular pathways.^{2,5,20} Cortisol also modulates immune function, acting as an anti-inflammatory agent and suppressing immune reactions.^{2,5,20} Teleologically, these actions may be of benefit in the response to training. Overload through increased exercise intensity, resistance, or duration is necessary for training to stimulate an adaptive response to exercise. The minor disruption of function and structure in the cells of the muscles results in protein accretion, substrate uptake, and deposition, and other beneficial remodeling that increases the functional capacity of the cells. Cortisol's suppression of immune function and anti-inflammatory effects may actually provide a permissive environment for tolerating the slight amount of 'muscle damage' needed for training-induced remodeling.

Many studies have demonstrated that cortisol is increased in the horse during a wide variety of exercise activities, from racing to polo to endurance rides.^{11,20,32,74–80} The release of cortisol in the horse appears to be affected by both intensity and duration of exercise.^{20,79,80} However, excessive increases in cortisol concentrations following exertion can be a marker of too much exercise. Prolonged cortisol recovery times as well as either inappropriately high or low plasma concentrations of cortisol may be markers of overtraining in the horse. As mentioned above, post-

exertion effects of cortisol may elicit a permissive effect beneficial for training adaptation by preventing the immune system from eliciting an inflammatory and immune reaction to acute exercise.^{11,81} Several studies of horses have followed cortisol levels for an extended period post-exercise.^{82–84} Those experiments demonstrated that exercise caused a sixfold increase in adrenal cortisol secretion and a 2–3-fold increase in plasma cortisol concentration. Urinary cortisol concentrations also increased threefold with a return to baseline levels by 10 hours post-exertion.^{82–84} The authors also noted a substantial increase in liver clearance of cortisol.

Interestingly, data are mixed as to the effects of training on the cortisol response.^{20,79,80} Studies have suggested that peak post-exercise cortisol concentrations are reached earlier in trained horses and that trained horses have a faster cortisol recovery time.^{20,79,80} On average, peak cortisol levels were observed at about 30 minutes post-exertion.^{20,79,80}

Pancreas

The pancreas is a V-shaped organ that lies along the duodenum.^{2,5,20} Structurally, most of the pancreas is composed of acini which function as exocrine cells, secreting digestive enzymes and bicarbonate into the small intestine via the pancreatic duct. The endocrine function of the pancreas is mediated by cells of the islets of Langerhans.^{2,5,20} These specialized cells are arranged as branching cords surrounded by a large network or plexus of capillaries.^{2,5,20} The cells are classified into three types: the α -cells which produce glucagon, the β -cells which produce insulin, and the δ -cells which produce somatostatin.^{2,5,20} Of those hormones, the most important during exercise are insulin and glucagon and their actions in the control of glucose metabolism.

Insulin

Insulin functions as part of the feedback system controlling blood glucose concentration.^{2,5,20} Insulin is synthesized by the β -cells of the pancreas and is primarily a glucose 'storage' hormone because it facilitates glucose uptake by the cells, promotes glycogenesis, and inhibits gluconeogenesis.^{2,5,20,85,86} At rest insulin is the 'key' that opens the cellular door to allow uptake of glucose.^{2,5,20} However, during exercise the working muscles take up glucose without insulin.^{5,20}

Thus, insulin is very important during the recovery from exercise when glycogen repletion is most active.^{2,5,20,87–89}

The insulin response to acute exercise has been well documented, with the horse, like humans and other species, suppressing insulin during exercise.^{20,76–78,80,90–96} This suppression appears to have a threshold of 50% of $\dot{V}O_{2\max}$ which coincides with the increase in catecholamines seen during exercise.^{1,20} Recent more mechanistic studies have demonstrated the link between exercise-induced increases in sympathetic drive and changes in insulin and glucagon secretion in the horse.^{95,96} Functionally this allows the animal to increase gluconeogenesis to maintain blood glucose concentrations during exercise.^{5,20,95,96} Glucose mobilized during exercise can be taken up by the muscles with insulin; however, endurance performance appears to be limited by central fatigue mechanisms more than peripheral fatigue.^{5,20,95,96} Suppression of insulin and maintenance of blood glucose concentration prevent the onset of central mechanisms of fatigue.^{6,73} Much of the recent work on the insulin response to exertion has centered on the composition and timing of pre-exercise feeding.^{74,97–105} High-carbohydrate feeds are beneficial for optimal muscle glycogen synthesis to fuel exercise; however, the resultant increase in blood glucose seen after a horse eats a high-carbohydrate ration usually evokes an increase in insulin secretion.^{102,104–106} The goal of recent research has been to prevent this feed-induced spike in insulin that would tend to decrease blood glucose directly before or during exercise.^{102,104–107} Training appears to alter the insulin response to exercise, enhancing the ability to synthesize glycogen during recovery.²⁰

Glucagon

The functions of glucagon are in opposition to insulin in that it stimulates gluconeogenesis and inhibits glycogenesis.^{2,5,20} Glucagon is one of many hormones needed for substrate mobilization and thus it increases during exercise in the horse.^{76–78,85,108,109} As such, glucagon is important for maintaining glucose concentrations during exercise, a role that is especially important during endurance activities where a drop in blood glucose concentrations leads to the onset of central fatigue.^{2,5,20} Published data from several studies have demonstrated that endurance horses have an increase in glucagon.^{76–78} This increase in glucagon is also altered by exercise intensity and its release appears to be under the influence of the

increases in sympathetic drive and the catecholamines.⁹⁶ Training appears to alter the glucagon response to exercise, enhancing the ability to mobilize glucose during exertion.^{5,20}

Other pancreatic hormones

Other hormones produced by the pancreas and associated with both endocrine and exocrine pancreatic function may play an important role in modulating substrate disposition during and after exercise.¹¹⁰ These hormones include pancreatic polypeptide, somatostatin of pancreatic origin, amylin, and galanin.¹¹⁰

Pancreatic polypeptide

Pancreatic polypeptide (PP) does not appear to affect insulin or glucagon concentrations. However, comparative studies have suggested that pancreatic polypeptide affects digestion.¹¹⁰ Hall and co-workers¹⁰⁹ point out that PP inhibits pancreatic exocrine function and bile secretion, an observation that they considered appropriate for a horse during long-term exercise when food intake would tend to be minimal. Information regarding the effect of exercise on PP is minimal. However, in one study Hall et al¹⁰⁹ demonstrated that PP increases in the endurance horse from concentrations averaging 20 pmol/L at rest to levels as high as 102 pmol/L after an 80 km ride. Lower concentrations seen after a 42 km race would suggest a dependence on duration which may be related to the degree of hypoglycemia seen in the horses post-exertion.¹⁰⁹ These results are similar to those seen in other species.¹¹⁰ We are unaware of any published studies that have examined the effect of exercise intensity in the horse.

Somatostatin

Somatostatin is produced in the hypothalamus, the gastrointestinal tract (see below), and the pancreas. It is well recognized that the somatostatin produced in the hypothalamic region of the brain inhibits somatostatin (i.e. growth hormone) as well as thyrotrophin release.¹⁰⁹ Somatostatin of pancreatic origin is produced by the δ -cells of the pancreas and functions locally, to alter pancreatic function, and regionally, possibly to alter blood flow and also restrict nutrient absorption.¹⁰⁹ Hall and co-workers¹⁰⁹ have suggested that this fits with the well-documented reduction in splanchnic blood flow that occurs during exercise. A small but significant increase in somatostatin concentration has been demonstrated during endurance

exercise per se with no difference due to duration (42 versus 80 km).¹⁰⁹ This is consistent with studies of humans and other species.¹¹⁰ Published studies of the horse have not examined the effect of exercise intensity on concentrations of somatostatin.

Amylin and galanin

Amylin and galanin are two other substances produced by the pancreas that affect pancreatic function and influence pathways involved in insulin regulation.¹¹⁰ Amylin is a 37-amino acid protein produced by the β -cells of the pancreas, whereas galanin is a 29-amino acid protein secreted by nerve cells in the pancreas.¹¹⁰ These paracrine substances may be influenced by exertion; however, we are unaware of any published studies in horses or other athletic species.

Circulating gastrointestinal ('gut') hormones

Several other substances with endocrine and paracrine function are secreted by the digestive tract.¹¹⁰ These substances alter digestive function and thus may influence digestion and the absorption of substrate during exercise.¹¹⁰ Included in this complex array of modulatory substances are gastric inhibitory peptide (GIP), vasoactive intestinal polypeptide (VIP), gastrin, somatostatin, secretin, enteroglucagon, motilin, cholecystokinin (CCK, see also below), the enkephalins, the endorphins, substance P, gastrin-releasing peptide, neuropeptide Y, peptide YY, and neurotensin.¹¹⁰

Gastric inhibitory peptide

This is a 42-amino acid peptide that has a dual action, inhibiting gastric acid production and stimulating insulin secretion.¹¹⁰ Exercise does not alter GIP in humans; however, consumption of glucose during recovery appears to result in decreased concentrations of GIP in humans. Hall et al.¹⁰⁹ reported that plasma GIP concentrations were not altered during endurance exercise (42 and 80 km). However, those results should be looked at with caution as the authors had relatively low animal numbers. The longer endurance competition did result in a non-significant decline in plasma GIP concentration from approximately 75 pmol/L before exercise to 50 pmol/L after 80 km.¹⁰⁹ The decline in GIP and its insulin secretagogue action would be consistent with the substantial and signifi-

cant suppression of insulin concentrations reported in those same horses.

Vasoactive intestinal polypeptide

Vasoactive intestinal polypeptide is a 28-amino acid peptide acting as a neurotransmitter that is secreted by nerve fibers in the GI tract.¹¹⁰ Multiple actions of VIP include vasodilation, stimulation of glucagon secretion, and enhancement of the stimulation of substrate release through lipolysis and hepatic glycogenolysis.¹¹⁰ Endurance exercise has a pronounced effect on VIP secretions, with increases in plasma concentrations that appear to be affected by exercise duration.^{109,110} Teleologically this fits with the energy substrate needs associated with prolonged exercise.

Gastrin

This is a 10-amino acid peptide that stimulates gastric acid secretion that is affected by prior ingestion of food.¹¹⁰ Limited data are available on the effects of exercise on the plasma concentration of this hormone in the horse.¹⁰⁹ Human studies¹¹⁰ have not reported increases in gastrin due to exercise but in a paper on horses by Hall et al.,¹⁰⁹ while plasma concentrations of gastrin were not altered by a 42 km endurance ride, they were substantially increased following a longer 80 km ride. One would presume, though, that the horses in the longer ride went for a longer time without food intake and, therefore, this increase in gastrin secretion would seem unwarranted. However, one wonders if this paradoxical increase contributes to excessive acid production and gastric ulcer formation when food is withheld from a horse for a long period of time.

Other gastrointestinal peptides with endocrine or paracrine actions

Most of these substances have not been studied in the horse; however, comparative studies have shown that they have localized action within the GI tract. Many of these substances have been characterized as neurotransmitters that act on local tissues, altering membrane transport, stimulating motility, and, in some cases, stimulating acid production.¹¹⁰ For example, gastrin-releasing peptide is a 27-amino acid peptide that is also referred to as bombesin.¹¹⁰ This protein stimulates gastrointestinal motility and the release of gastrin.¹¹⁰ Secretin and enteroglucagon are both 29-amino acid hormones that inhibit acid secretion in the

stomach.¹¹⁰ Comparative studies have shown that there is an increase in peripheral blood collected after exercise in both humans and dogs.¹¹⁰ Motilin has been shown to increase with exercise in humans.¹¹⁰ This 22-amino acid peptide stimulates motility of the gastrointestinal tract.¹¹⁰ Interestingly the enkephalins appear to have the opposite effect, decreasing motility.¹¹⁰ Other peptides like substance P, neuropeptide Y, and peptide YY appear to alter GI tract motility but little is known about changes during exercise.¹¹⁰ Interestingly, the functional link between all the GI tract hormones appears to be their actions on motility and possibly transport.¹¹⁰ These actions may make them important for the uptake of water, electrolytes, and energy substrates during and after exercise.¹¹⁰ Finally, neurotensin is a 10-amino acid GI hormone with an unknown role; however, studies have shown that it increases during exercise in humans when a glucose solution is consumed but not when water alone is consumed.¹¹⁰

Hormones related to appetite and energy balance

Maintaining energy balance is crucial for the optimal health and performance of exercising horses. The energy expended during exercise directly affects energy homeostasis, because the horse has to increase energy intake in order to compensate both for the energy lost during exercise and for the energy required for the recovery and repair of tissues. Although the neuroendocrine control of energy balance has been studied extensively in humans and rodents, it is just beginning to be examined in horses. Examples of some of the endocrine mediators measured to better understand the control of energy balance are the hormones leptin, adiponectin, ghrelin, and cholecystokinin.

Leptin

Leptin is an adipocyte-derived hormone, a 16 kDa protein product of the *ob* gene, that acts as an indicator of energy balance.^{111–113} Sensed levels of leptin influence neural transmission in brain pathways, affecting food intake and energy utilization.¹¹⁴ Basically, high levels of leptin increase energy expenditure while decreasing food intake and vice versa.¹¹⁴ Food intake is altered by leptin influencing responsive neurons in the brain that activate either a feeding or satiety system.¹¹⁴ The feeding or orexigenic system contains neuropeptide Y, agouti-related protein, and

other hormones and neurons that signal an animal to increase food intake, while the satiety system involves neurons containing pro-opiomelanocortin and α -melanocyte stimulating hormone as well as others that decrease food intake.¹¹⁵ Leptin increases energy expenditure by stimulating the sympathetic nervous system in brown adipose tissue, directly increasing the expression of uncoupling protein 1, and by possibly increasing the expression of uncoupling protein 3 in muscle.¹¹⁶ Furthermore, leptin strongly stimulates triglyceride and fatty acid cycling by increasing lipolysis and fatty acid oxidation.^{117,118} Leptin is secreted in proportion to fat mass,^{117,118} although massively obese humans seem to be 'resistant' to leptin's slimming propensities.^{111,119}

In horses, plasma leptin is positively correlated with percent fat mass and body condition score.^{120,121} Leptin has also been found to have a seasonal variation in both young and old mares, with plasma leptin levels increasing in the summer and decreasing in the winter, in correlation to bodyweight and percent fat mass.¹²² Furthermore, 24-hour fasting decreases plasma leptin levels in young and mature mares.¹²³ Interestingly, one study showed serum concentrations of leptin were higher in geldings and stallions versus mares, which is incongruent with human literature in which females have higher leptin levels than males, with differences not completely explained by a greater percent fat mass in females.^{121,124} In rats, recent research demonstrated that male rats had higher leptin concentrations in the blood than female rats.¹²⁵ The reason for the discrepancy between species is unclear.

With regard to exercise, there have been some interesting findings in humans on how the type and duration of exercise affect energy balance and leptin concentrations in the blood. To date, no such studies have been published in horses. In humans, however, studies involving short-term exercise (<60 min) with varying intensities have generally shown no change in leptin concentrations due to exercise.^{126,127} Studies that have reported changes in leptin concentration with short-term exercise have attributed the changes to hemoconcentration or circadian rhythm.^{128,129} It is possible that short-term exercise does not cause a sufficient kilocalorie deficit to produce a disruption in long-term energy balance. Also, the interaction between other hormones (e.g. cortisol, insulin, glucose, epinephrine (adrenaline) and norepinephrine (noradrenaline)) that fluctuate during exercise and have been found to either stimulate or inhibit leptin secretion remains to be determined.^{1,129,130}

Long-term exercise (≥ 60 min) of varying intensities has been shown to decrease or cause no change in leptin concentrations.^{131–133} Interestingly, studies showing reductions in leptin increased sampling times for up to 48 hours after exercise, with a reduction in the 24-hour mean and amplitude of the circadian rhythm of leptin.^{130,134} It appears that long-term exercise, in well-controlled studies, provides enough of an energy deficit to decrease leptin levels, which in turn will increase food intake to help maintain energy balance.¹³⁵

Training is another aspect of exercise that is of interest to scientists studying leptin concentrations and energy balance. Training regimens have had different effects depending on duration and intensity of exercise and subjects used. Briefly, training for less than 12 weeks generally causes no change in leptin levels, although type II diabetic individuals did show a reduction in leptin concentrations after 6 weeks of low-intensity walking and cycling, independent of body composition changes.^{136,137} Training regimens of longer than 12 weeks can cause a reduction in fat mass, which lowers leptin levels, yet some studies report a reduction in leptin independent of fat mass changes.^{138–140} Additionally, it appears that females are more sensitive to the training effect on leptin levels, with several studies showing female subjects, yet not their males counterparts, demonstrating lower leptin concentrations in response to training.¹⁴¹

Leptin has several potential roles in terms of the health of exercising horses. As a signal of energy homeostasis, leptin concentrations in horses can help scientists to determine if a horse is in positive or negative energy balance and can provide supportive data regarding a horse's body condition and percent fat mass. Negative energy balance is detrimental to exercise performance, reproductive status, and overall health.¹⁴² Furthermore, determining how exercise and training affect leptin concentrations in horses will allow better understanding of how horses regulate their energy balance so that training regimens and diets can be adjusted accordingly to optimize the health of the athlete.

Adiponectin

Adiponectin is another hormone secreted from adipocytes, with its role in metabolism related to the regulation of glucose, insulin, and adipocyte metabolism.^{143–145} In contrast with leptin, adiponectin levels

are decreased in obese and insulin-resistant humans and animals.^{146,147} With regard to exercise, adiponectin may have a role in the increased insulin sensitivity seen as a result of training in both humans and horses.^{148–151} In a study of humans undergoing exercise training, however, plasma adiponectin concentrations did not change relative to the increased insulin sensitivity due to training.¹⁵²

In horses, adiponectin is negatively correlated with percent fat mass in yearling fillies and mature mares.¹²⁰ The study of adiponectin in horses is of importance as it is likely related to the insulin resistance commonly seen in older horses and horses with pituitary adenomas. Studies have shown that older mares with impaired glucose tolerance were able to improve their insulin sensitivity with 12 weeks of training.¹⁴⁹ It would be of interest to determine if adiponectin has a role in this insulin-sensitizing phenomenon.

Ghrelin

Another hormone involved in the control of appetite and energy balance is ghrelin, a protein hormone secreted from the stomach which was first discovered as a potent growth hormone secretagogue.¹⁵³ Ghrelin has received most of its attention, however, due to its role in initiating food intake in humans and rodents.¹⁵⁴ In meal-fed animals, including humans, rodents, and sheep, ghrelin increases before meal feeding in anticipation of the meal and will also increase during times of fasting.^{155–157} In rats, ghrelin stimulates gastric acid secretion in the stomach.^{158,159} Few studies to date have examined the role of ghrelin in relation to exercise, in any species. One study in humans, however, demonstrated that ghrelin levels did not change during submaximal aerobic exercise in healthy adults,¹⁶⁰ although the paper was focused on the relationship between ghrelin and growth hormone released during exercise and not that between exercise, ghrelin, and food intake.

It would be valuable to attempt to study ghrelin in horses as it may have a significant role in helping horses to maintain energy balance. In addition, high-performing equine athletes often have problems with inappetence and gastric ulcers that may be related to abnormal ghrelin concentrations and ghrelin's stimulation of gastric acid.¹⁶¹ Humans with anorexia exhibit higher ghrelin concentrations than their normal counterparts, with a presumed 'ghrelin resistance' contributing to the cachexia of this eating disorder.^{162,163}

Cholecystokinin

The peptide hormone cholecystokinin (CCK), secreted from the small intestine, is involved in energy balance by signaling fullness and decreasing food intake in humans, rodents, and ruminants.^{164–167} To date, there has been little published data on CCK in horses but this hormone may play a role in the inappetence commonly seen in heavily exercised horses. In a study conducted in humans, exercise increased plasma CCK concentrations fourfold. Although CCK values returned to normal at the end of exercise, equine researchers have speculated that CCK concentration may increase in response to exercise in horses and remain elevated, contributing to a lack of interest in feed by some equine athletes.¹⁶⁸

Finally, it must not be ignored that there is also an interaction between many of the above-mentioned hormones in relation to energy balance. For example, CCK enhances the effect of leptin administration on weight loss and the pair may directly decrease food intake.^{169,170} Leptin and adiponectin, although expressed in opposite concentrations to one another, may be regulated similarly for short-term alterations, yet differently for long-term regulation.¹¹² Ghrelin, on the other hand, is upregulated during leptin therapy, although these increases in ghrelin are not able to overcome the food intake depression caused by leptin.^{171,172} Hence, it is clear that these endocrine mediators should not just be studied in isolation but collectively to determine how they regulate various systems.

In conclusion, the regulation of energy balance in horses and how it is affected by exercise is a field that has yet to be investigated in depth. On the other hand, data published in humans and other species demonstrate the importance of such research, especially with regard to gastric ulcer syndrome and the inappetence commonly seen in heavily exercised horses, and obesity and insulin resistance seen in many older horses. It is hoped that future research in this field will elucidate the characterization of energy balance in horses, how this equanimity is maintained in response to exercise, and ways in which management practices can be changed to help horses remain in energy balance and achieve optimal performance and health.

Kidneys

Filtration of the blood and conservation of vital substances are the most obvious functions of the

kidneys.^{40,61} However, their close link with the control of cardiovascular function is more complex and multifaceted. The basic filtration unit is the kidney glomerulus.^{40,61} Each of these glomeruli has a specialized group of cells referred to as the juxtaglomerular apparatus (JGA),^{40,61} which has more specialized cells that act as feedback sensors in monitoring flow (and pressure), sodium and chloride concentration, and arterial PO_2 .^{40,61} The major hormone produced by the kidney is renin, the activating substance in the renin–angiotensin–aldosterone cascade which has the potential to alter blood pressure, volume, and tonicity.^{40,61} The kidney also produces erythropoietin which acts on precursor cells in the bone marrow to stimulate red blood cell production. Both of these hormone systems play an important role in the defense of normal cardiovascular function.^{40,61}

Plasma renin activity (PRA)

Renin is a hormone released by the JGA of the kidney. It facilitates the conversion of angiotensinogen into angiotensin I, which is converted in the lung to angiotensin II, a vasoconstrictor that also stimulates the production and release of aldosterone.^{38–40,45,173} During exercise, PRA is a measure of the rate of angiotensin I generation.^{38–40,45} Angiotensin I and angiotensin II are powerful vasoconstrictors involved in the control of MAP and blood flow during exercise.^{38–40} After exercise, renin can directly affect renal function and angiotensin stimulates thirst and drinking, thus altering post-exercise fluid balance.^{38,39,41,45,174} Three major mechanisms that may account for the increase in PRA during exercise are renal nerve stimulation via increased sympathetic drive, changes in renal blood flow and pressure associated with juxtaglomerular function, and changes in electrolyte (sodium and chloride) concentrations at the JGA in the kidney.^{38–40,45,173}

Previous studies have measured PRA in horses at rest, after exercise training, during steady-state exercise, or after brief maximal exercise.^{42,45,61–65,68} A strong linear correlation exists between work intensity (and duration) and increases in PRA, and HR were reported up to treadmill speeds of ~9 m/s.⁴⁵ Above 9 m/s, heart rate (HR) and PRA reached a plateau and did not increase when speed was increased from 9 to 10 m/s.⁴⁵ In previously published studies of horses, PRA increased from 1.9 ± 1.0 ng/mL/h at rest to a peak of 5.2 ± 1.0 ng/mL/h at 9 m/s.⁴⁵ The observed concurrent plateau in PRA and HR supports the suggestion that the increase in PRA during exercise in the

horse is linked to sympathetic drive. Such is the case in other species where mechanistic studies have demonstrated a correlation between renal sympathetic nerve activity and PRA.^{38–40,173} During steady-state submaximal exercise, the major factor stimulating an increase in PRA early in exercise was an increase in sympathetic drive.^{38–40,61} However, a secondary increase in PRA was seen in horses after 40 min of exercise and was most likely due to a decrease in plasma Cl^- concentration, which fell significantly during this period, and not plasma Na^+ , which remained constant.⁴²

Functionally, an increase in PRA during exertion has been shown to result in an increased plasma angiotensin II concentration.^{38–40,61} Interestingly, horses given the angiotensin-converting enzyme inhibitor, enalapril, had significantly lower plasma angiotensin II, aldosterone concentrations, and pulmonary artery pressures during exercise compared to horses given a placebo.⁶⁸ These latter observations demonstrate that the renin–angiotensin cascade plays a role in the control of blood pressure during exercise in the horse.

Erythropoietin

Erythropoietin (EPO) is a peptide hormone that is produced by the kidneys in response to hypoxia sensed by pericellular cells positioned in the vasculature of the renal matrix.^{175–178} Recent papers have documented the effects of a variety of perturbations, including the effects of blood loss, acute exercise, and altitude, on EPO production in humans.^{175,176,179} If cardiopulmonary adjustments are insufficient to prevent hypoxemia and if the above-mentioned perturbations are large enough to cause a decrease in renal arterial partial pressure of oxygen (PaO_2), then plasma EPO concentrations increase and there is a subsequent stimulation of erythropoiesis in humans.^{175,176,179} The resultant increase in red blood cell volume would be expected to couple with other compensatory mechanisms to return arterial PO_2 back up to normal levels.

McKeever et al¹⁸⁰ recently reported that acute exercise does not appear to stimulate an increase in plasma EPO concentration in normal horses. This is similar to observations made in several studies of humans, which demonstrated that neither the intensity nor the duration of acute exercise alters plasma EPO concentrations in humans.^{181–183} Teleologically, this makes sense because one would speculate that if acute exercise caused substantial increases in EPO production and release, then repeated exercise (i.e. training)

would cause a sustained increase in EPO.¹⁸⁴ Any such hypothetical repeated increase in circulating EPO concentration would be expected to cause a sustained stimulation of erythropoiesis. Taken a step further, this would eventually cause a red cell hypervolemia and potentially detrimental increases in blood viscosity. Mechanistically, the acidosis of exercise appears to inhibit EPO production.¹⁸⁵

Altitude appears to cause a transient increase in plasma EPO production in humans and horses.^{5,180,186,187} However, in horses, plasma EPO concentrations increased only during the first 3 hours of the first day at 3800 meters.¹⁸⁰ This is similar to studies of humans, which have reported a ‘temporary rise’ in EPO concentrations in mountaineers at both 4900 and 7600 m.¹⁸⁶ One explanation for this rapid return to baseline concentrations is a rapid compensation of cardiorespiratory mechanism to the challenge of altitude that would tend to limit changes in PaO_2 at the kidney.¹⁸⁶ Interestingly, exercise performed at altitude did not induce a secondary increase in plasma EPO concentration.¹⁸⁰ The authors concluded that hypobaric hypoxia positively affects EPO production in the horse rapidly upon the first day at altitude with a rapid return to pre-altitude concentrations.¹⁸⁰ Their data suggest that horses have an innate ability to tolerate the acute challenges induced by exercise and altitude.

Interestingly, administration of recombinant human erythropoietin (rhEPO) has been shown to increase hemoglobin concentration and exercise capacity in humans with chronic renal failure.^{188–190} Small doses of rhEPO have been found to increase hemoglobin concentration by 30% and increase endurance performance anywhere from 10% to 19% in healthy human subjects.^{188–190} Purportedly, some human athletes and/or their trainers have decided to use higher than recommended doses of erythropoietin with the rationale that the more rhEPO injected, the greater the increase in aerobic capacity.^{188–190} Injections of rhEPO have been shown to elevate resting hematocrit to levels greater than 55% in humans, which increases blood viscosity and clotting and enhances the risk for heart attack or stroke.^{188,190} Rises in hematocrit increase blood viscosity and may have caused excessive increases in blood pressure and clotting problems related to the deaths of human athletes in Europe.^{188,190}

Unfortunately, these practices have entered equine sports medicine, with clinicians, horse trainers and racing commission personnel reporting that this drug is being misused in race horses to improve

performance through an increase in blood volume and oxygen-carrying capacity.^{178,191,192} However, two major problems can develop in horses.^{175,178,193} First, while the horse can tolerate hematocrits of 50–60% during exercise, no one knows what happens to a horse's cardiovascular system if the normal resting hematocrit is artificially elevated to values of 70–80%.^{178,193} A large increase in resting hematocrit coupled with splenic reserve mobilization may produce dangerously viscous blood that may lead to sudden death during or after exercise.¹⁷⁸ If this increase in resting red blood cell volume were to be coupled with lasix-induced fluid losses, the results could be devastating.¹⁷⁸ A second major potential problem associated with rhEPO misuse in the athletic horse is its reactivity with the horse's immune system.^{178,193} Some horses have purportedly developed a life-threatening anemia associated with an immune reaction to both the exogenous rhEPO and the animal's own EPO.^{178,193} Clinically, resting hematocrit has been seen to drop below 20% in horses reacting to rhEPO administration, with some cases requiring blood transfusions to save the horse's life.¹⁹³

Recently, studies of splenectomized and intact horses have demonstrated that rhEPO administration in low doses causes substantial increases in resting hematocrit, red blood cell volume, maximal oxygen uptake, blood viscosity, and selected hemodynamic variables during incremental exercise performed on a treadmill.^{179,194} In splenectomized horses, administration of rhEPO in low doses (15 IU/kg) three times a week for 3 weeks increases resting hematocrit from 37% up to 46%.¹⁹⁴ The resulting 13% increase in red cell volume was associated with a 19% increase in $\dot{V}O_{2\max}$ and substantial increases in blood viscosity.¹⁹⁴ Another study of intact horses also demonstrated that low-dose administration of rhEPO (50 IU/kg, three times/wk for 3 wks) increases red blood cell volume, $\dot{V}O_{2\max}$, and the velocity at $\dot{V}O_{2\max}$.¹⁷⁹ Viscosity was not measured in that study; however, post-exercise hematocrits were in the low 70s and considered dangerously high.¹⁷⁹ Horses in that study also developed antibodies to the rhEPO (McKeever, unpublished data).

Heart and blood vessels

The heart and blood vessels play both a paracrine and endocrine role in the control of cardiovascular function.³ While there are several mechanisms worthy of discussion, two hormones appear to play a major role

during exercise: atrial natriuretic peptide and the endothelins.³

Atrial natriuretic peptide (ANP)

This is a hormone produced by the heart that is important in the regulation of blood flow and blood pressure during exercise.^{3,195} Granules of ANP are stored within the walls of the atria and are released during atrial stretch.^{3,196} Receptor sites for ANP have been identified in the posterior pituitary, the kidneys, vascular smooth muscle, adrenal cortex, heart, and lung.^{3,197} This hormone causes a rapid and profound vasodilation and a pronounced natriuresis.^{3,197} ANP inhibits vasopressin, renin, and aldosterone secretion and also inhibits the binding of aldosterone at the kidney tubule.^{3,197}

On a practical level, ANP may be involved in accommodating the exercise-related shifts of blood volume in the horse.^{3,44} Evidence for this is provided by two recent studies which demonstrated that plasma ANP increases in a linear fashion with increasing work intensity, from 5–10 pg/mL at rest to plasma concentrations exceeding 60 pg/mL at speeds eliciting $\dot{V}O_{2\max}$.^{3,197–199} Mean ANP concentration was strongly correlated with heart rate.¹⁹⁷ Furthermore, ANP increases during steady-state submaximal exercise, from ~10 pg/mL at rest to a peak of 40 pg/mL at 40 min, and remained elevated through 60 min of exertion.^{3,42,200} Nyman and co-workers¹⁹⁹ presented similar peak plasma ANP concentration during steady-state exertion and reported that ANP concentrations were altered by hydration status. Horses that were hyperhydrated had the highest ANP concentrations during exercise compared to control and hypo-hydrated horses.¹⁹⁹ Another study found no differences between arterial and mixed venous ANP concentrations, suggesting that ANP either is not metabolized by the lung or is released from the left atrium at a rate matching pulmonary metabolism.²⁰¹ Even more recent work has examined the effects of exercise on ANP, with a special focus on its interaction between fluid and electrolyte status and other endocrine responses.^{69,202} The authors concluded that ANP remains elevated post-exercise and that this is a response to the exercise-induced increase in circulating blood volume rather than an interaction with vasopressin and the catecholamines.⁶⁹

Endothelin

The endothelins are peptide hormones that have pronounced effects on neuroendocrine control of

cardiovascular function.^{203–206} The endothelins, ET-1, ET-2, and ET-3, are isoforms of a 21-amino acid polypeptide with pronounced effects on both central and peripheral control of cardiovascular function.^{203–206} The three sequences of endothelin are structurally and pharmacologically distinct, arising from what has been called 'big endothelin', a 39-amino acid precursor molecule.^{203–206} The half-life of ET-1 is very short, only a few minutes, which is consistent with its role in control of vascular tone.^{205,206} Factors affecting the release and metabolism of endothelin include increased blood flow, vasopressin, angiotensin, shear stress, and thrombin.^{205–213}

Many studies have shown that ET-2 and ET-3 are limited in their vascular effects and that ET-1 has the most pronounced effect on peripheral vascular tone.^{205,206,212,213} Endothelial cells produce ET-1 exclusively²⁰⁵ and circulating levels of this hormone may play a role in certain forms of hypertension.^{205,206,214} ET-1 and ET-2 (to a minor degree) are potent vasoconstrictors that can increase systemic arterial blood pressure and pulmonary arterial blood pressure and cause alterations in cardiac output and the distribution of blood flow in the peripheral circulation.^{205,206} Thus, ET-1 may affect the redistribution of blood flow and control of blood pressure during exercise. ET-3 appears to play a role in modulating the release of vasopressin from the hypothalamus, and Rossi²⁰⁵ has shown that ET-3 amplifies free water excretion independent of renal and systemic hemodynamic and osmotic clearance and/or circulating vasopressin concentrations.

Resting plasma concentrations of immunoreactive ET-1 measured in horses appear to be similar to the relatively low concentrations reported for other mammalian species such as the rat, dog, pig, cow and man,^{205,206,210,213,215,216} and horses.^{198,204,217–220} Studies of ET-1 in horses have focused primarily on understanding its role in horses with either respiratory disease^{217–220} or aging.^{198,204} In some of those studies, ET-1 was found to be elevated in blood and bronchiolar alveolar lavage fluid in resting horses with respiratory disease.^{218,219} Many recent experiments have only reported on samples obtained either before and after exertion^{218,219} or at rest and at the speed eliciting $\dot{V}O_{2\text{ max}}$.²⁰⁴ However, one recent study has examined plasma ET-1 concentrations in the horse during exercise rather than just collecting blood samples before and after exercise.²⁰⁴ Samples taken while the horses were running a graded exercise test (GXT) revealed that during exercise there were no changes in plasma ET-1 concentration and there were

no alterations due to increases in work.²⁰⁴ Interestingly, while plasma ET-1 concentration did not change with increases in exercise intensity, it did increase substantially in samples collected immediately after the exercise stimulus was withdrawn and in blood collected 2 min following cessation of running.²⁰⁴ However, plasma ET-1 concentrations were back to normal by 10 min.²⁰⁴ This rapid response may be physiologically significant as it coincides with the rapid recovery and transient decreases in cardiovascular function reported in other studies of horses that have involved protocols with a quick stop of the treadmill.^{3,68}

Similar post-exertion increases in plasma ET-1 concentration have also been reported in studies of normal humans and in studies where the subjects had various diseases affecting the vasculature.^{211–213,221} In one of those experiments even greater increases in plasma ET-1 concentrations were seen in dehydrated humans.²¹⁶ The greater increase in plasma ET-1 concentration due to volume depletion supports the suggestion that ET-1 plays a role in the modulation of vascular tone in the defense of MAP.²⁰⁶ Thus, the post-exertion increase in plasma ET-1 concentration observed in horses²⁰⁴ sampled before and after exercise is consistent with previously published reports on the hemodynamic and endocrine responses to exercise in horses.^{3,8,62} An increase in plasma ET-1 concentration following rapid cessation of prolonged exercise, at a time when post-exertion blood pressure would be expected to fall, fits with the neuroendocrine response to other perturbations affecting vascular fluid volume, cardiac filling pressure, and MAP. This would be an appropriate response in the regulation of cardiovascular function during the transition from exertion to recovery when heart rate, cardiac output, and blood pressure are declining rapidly in the face of exercise-induced vasodilation of vascular beds supplying the muscles.⁶⁴

A great deal of information has been published documenting elevated resting plasma ET-1 concentrations in humans and rats with diseases, in particular in humans with chronic obstructive pulmonary disease (COPD) and pulmonary hypertension.^{203,206,210,211,213–223} Data reported by Benamou et al²¹⁸ demonstrated that post-exercise ET-1 concentrations were substantially elevated in the bronchoalveolar lavage fluid of horses with EIPH. In another study Benamou et al²¹⁷ demonstrated that ET-1 type A receptors mediate the vasoconstrictor action of ET-1 in the pulmonary and systemic circulations of the horse. However, data from another study²²⁰ suggest

that ET-1 is not a mediator of the acute hypoxic pulmonary hypertension response to exercise, but may serve as a modulator of the acute response or slower phase of hypoxic pulmonary hypertension response to exercise. More work is needed to determine if ET-1 plays a role in the etiology of exercise-induced pulmonary hemorrhage (EIPH), especially since some feel that increases in pulmonary artery pressure during exercise may contribute to EIPH.^{223,224}

Gonads and reproductive hormones

The reproductive hormones are essential for the health and well-being of a mare or stallion. However, exercise performance is not affected per se by the reproductive hormones. Nevertheless, recent human research has focused a great deal of attention on the effects of exercise on the female reproductive cycle and the interaction of prolactin, LH, FSH, estrogen, and β -endorphin.⁵ Human work has also examined the effects of acute exercise on the health of pregnant women. More work is needed in the horse to determine if exertion affects these hormones, especially in endurance horses and in pleasure horses that are ridden while they are in foal.

Endocrine mediation of short-term control of cardiovascular function

The cardiovascular response to exercise is dependent on a multisystem defense of blood volume, MAP, and plasma tonicity.³ These mechanisms insure adequate blood flow to the working muscles and obligate tissues along with the provision of adequate fluid volume for sweating and thermoregulation.^{1,3,5,38,39,41} The maintenance of cardiovascular homeostasis during exercise is mediated by neuroendocrine mechanisms which insure the system can meet the increased demand for blood flow to the working muscles during exercise.^{1,3,38,39,41}

The anticipation of exercise in humans and horses can invoke a withdrawal of parasympathetic control and an increase in sympathetic nervous activity resulting in an increase in heart rate, force of contraction, stroke volume, and cardiac output. Rowell⁴ suggests that the 'range of parasympathetic control of the heart by central command determines the level of exercise at which the activation of the sympathetic

nervous system occurs.' In horses, resting HR averages 30–40 bpm.³ Initial increases in HR up to ~120 bpm are associated with the withdrawal of parasympathetic tone.³ However, further increases in HR during exercise, up to maximal HR between 200 and 240 bpm, are associated with increases in sympathetic activity and catecholamine release.³ This increase in HR, coupled with increased stroke volume from 0.7 L at rest to almost 2 L, results in a rise in cardiac output from an average of 30 L/min at rest up to nearly 300 L/min during maximal exercise.³ The cardiovascular system responds to exercise with dramatic increases in heart rate and force of cardiac contraction and subsequent increases in stroke volume and cardiac output.⁴ These central cardiovascular responses are rapid and concurrent with venoconstriction and arterial vasodilation in the working muscles.⁴

Adjustments in peripheral vascular resistance that are mediated by the cardiopulmonary baroreflex cause a redistribution of blood volume from 'storage' in highly compliant venous capacitance vessels into the arterial side of the cardiovascular system, enhancing venous return.⁴ In the horse there is the added component of splenic reserve mobilization which further enhances venous return and circulating red cell volume.^{3,58} Splenic contraction is mediated by direct stimulation from the sympathetic nervous system, through the action of norepinephrine (noradrenaline) and epinephrine (adrenaline) on α -adrenergic receptors.⁵⁸ From 6 to 12 liters of blood can be delivered into the central circulation at the onset of exercise, allowing the equine athlete to reach a maximal aerobic capacity (145–200 mL/kg/min in fit horses) that is almost three times greater than that of human athletes.^{3,58} This extra volume is rapidly accommodated through arterial vasodilation, which is mediated by increases in sympathetic neural outflow and ANP and through local chemoreceptor mechanisms.^{3,44} Resequstration of the splenic reserve is prevented by the action of vasopressin and the catecholamines on splenic arterioles.⁴⁶

Mean arterial blood pressure increases with exercise intensity, a response essential for increasing cardiac output in the face of decreases in resistance in the vascular beds of the working muscles. Rowell⁴ suggests that in addition to input from the cardiopulmonary baroreceptors, a functional arterial baroreflex and muscle chemoreflexes are essential for the regulation of heart rate, cardiac output, and arterial pressure during exercise. Rowell⁴ further suggests that the operating point of the arterial baroreflex is 'reset

during dynamic exercise with adjustments in autonomic tone to compensate for the mismatch between cardiac output and vascular conductance.'

Modulation of the blood flow and blood pressure response to exercise involves input from both the high- and low-pressure baroreceptors. The low-pressure (cardiopulmonary) baroreceptors are volume receptors located primarily within the atria and the pulmonary circulation.^{3,4} At the start of exercise, increased venous return results in atrial stretch, eliciting a neuroendocrine response by the cardiopulmonary baroreceptors. Nerves within the atria serve as stretch receptors, sensing volume overload or underload. The output from these nerves is conducted centrally via vagal afferents and integrated into the central control of peripheral vascular tone.^{3,4} The endocrine component of this baroreflex involves the release of ANP, a hormone with potent vasodilatory properties, and a reflex decrease in vasopressin release.^{3,195}

Even with the mobilization of reserve blood volume, the demands of high-intensity or long-duration exercise may exceed central cardiovascular capacity. Baroreceptor control of arterial tone becomes vital to the maintenance of cardiac output and MAP, and sympathetic-induced vasoconstriction decreases blood flow to non-obligate tissues during high-intensity exercise.^{3,4} This response is even more pronounced during long-term exercise when fluid losses associated with sweating compromise vascular fluid volume and venous return. Without replacement or compensation, decreases in venous return associated with fluid losses can cause a decrease in cardiac output and decreased blood flow to the working muscles and to the vascular beds associated with thermoregulation.^{3,4,41} To maintain MAP, the body compensates by increasing HR and contractility, a phenomenon termed cardiovascular 'drift' that is associated with an increase in sympathetic activity and circulating catecholamines.⁵⁷

Exercise training produces chronic adaptations in the cardiovascular system that are mediated through changes in neuroendocrine control.^{3,37,38,41} Work from several species has shown that exercise training produces an expansion of plasma volume.^{41,65,72,225} Trained horses have significantly greater blood volumes than untrained horses^{41,58,65,72} with increases in plasma volume of 30% observed after only 1 week of exercise training. Humans show significant alterations in sodium and water excretion that are attributable to repeated exercise-induced increases in plasma aldosterone concentration.⁴¹ In one study of the

hypervolemic response in horses, the authors reported no change in resting plasma aldosterone concentration or sodium excretion; however, their measurements were taken at 1-week intervals and may have missed changes in sodium excretion that are reported to occur in the first days of training in humans.⁶⁵ A more recent study that focused on changes occurring during the first days of training demonstrates that plasma aldosterone concentration remains elevated for almost 24 hours during the first days of training.⁷² It appeared that, as in humans, an aldosterone-mediated retention of sodium and water by the kidneys and digestive tract is a vital part of the hypervolemic response to training in the horse.^{41,72}

Endocrine control of metabolism during acute exercise

Performance of exercise requires the transduction of potential or stored energy into kinetic energy, and the endocrine system plays an integral role in the coordination of the mobilization and utilization of carbohydrates and free fatty acids.^{226,227} The need for a rapid provision of metabolic substrates to fuel exercise and to prevent central fatigue is facilitated by a rapid increase in sympathetic drive and the rate of catecholamine release from the adrenal medulla. The degree of this response is correlated with both exercise intensity and duration.^{2,5,20} At the onset of exercise the catecholamines (epinephrine (adrenaline) and norepinephrine (noradrenaline)) act on the liver and muscles to increase the rate of glycogen breakdown (glycogenolysis).^{2,5,20} This results in an increase in circulating blood glucose concentrations. The catecholamines also stimulate the release of hormone-sensitive lipase which acts on triglycerides to mobilize free fatty acids.^{2,5,20} The latter are important for endurance activities where the use of fat to fuel exercise spares glycogen by offsetting the amount of glucose needed to fuel the activity.^{2,5,20} However, fat cannot be used alone as a fuel source as 'fat burns in the flame of carbohydrates.' An increase in circulating catecholamines also inhibits insulin and stimulates glucagon release. As with the catecholamines, glucagon also stimulates gluconeogenesis and inhibits glycogenesis,^{2,5,20} thus playing an important role in maintaining blood glucose concentrations during exercise and delaying the onset of fatigue.^{2,5,20} Glucagon can also

stimulate the breakdown of protein and release of amino acids which can be used as a fuel source by the liver.^{2,5,20} The effects of the catecholamines and glucagon can be augmented by the release of cortisol. The latter is affected by the intensity and duration of the activity.^{2,5,20} Cortisol thus is a metabolic hormone that

stimulates gluconeogenesis, fatty acid mobilization, and protein breakdown. In the case of the latter, amino acids not used to fuel exercise may provide resources for the synthesis of new proteins needed to repair muscle and replace enzymes used in the various metabolic pathways.^{2,5,20}

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Nutritional management of the equine athlete

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The owners, trainers, and riders of horses engaged in competitive athletic events are motivated to apply management strategies that provide a performance advantage during competition. Such strategies may include novel nutritional interventions administered before or during an event. Although there may be merit in some of these approaches, more realistically nutrition has a larger impact on performance by supporting consistent conditioning and thereby promoting the physiologic and biochemical adaptations that will ultimately result in improved athletic performance. For any athletic horse, the primary goals of nutritional management should include:

- provision of substrates for maintenance of bodyweight and replenishment of energy reserves in working muscle and other tissues
- promotion of tissue adaptation, growth, and repair
- promotion of general health and well-being
- application of competition feeding strategies appropriate to the athletic task.

Although these same general principles apply to all equestrian sports, actual feeding strategies may vary widely because of the different demands of training and competition. For example, whereas the consumption of a high-fiber (roughage) diet may be of benefit to an endurance horse via promotion of a large fluid reservoir in the hindgut, this strategy is not desirable in a race horse because excess gut fill may be energetically disadvantageous during high-intensity exercise. Similarly, the rationale for nutritional intervention immediately before and/or during competition will differ between disciplines. For an endurance athlete, feed consumption before and during a race may enhance energy supply and delay the onset of fatigue. On the other hand, for a race horse the consumption of a meal within 1–2 hours of the race start is less

likely to impact energy use and athletic performance substantially.

This chapter provides an overview of the nutritional needs of athletic horses with suggested feeding strategies to meet these requirements. The dietary management of horses with chronic exertional rhabdomyolysis and the putative role of ergogenic feeding strategies and nutritional supplements are also discussed.

Nutrient goals

The overall goal of diet formulation is to develop a ration that delivers optimal amounts of all essential nutrients. The 2007 edition of the National Research Council's (NRC) *Nutrient requirements of horses* provides nutritional recommendations for all classes of horse, including those undertaking regular physical activity.¹ However, it is important to realize that these recommendations are considered to be minimal rather than optimal requirements. Thus, while the 2007 NRC recommendations provide a framework for ration formulation and evaluation, many nutritionists have developed their own set of nutrient standards that, based on practical experience, provide optimal ranges of nutrients relative to the horse's physiologic state.

Energy

Undoubtedly, the most profound nutritional effect of regular physical activity is an increase in dietary energy needs relative to non-working horses. For bodyweight to be maintained, athletic horses must consume enough energy to cover 'maintenance' energy costs together with the energy costs associated with physical activity and the building and repair of muscle tissue. There are two primary considerations

with respect to dietary energy in athletic horses: provision of sufficient energy to maintain bodyweight and condition; and the sources of energy (fiber and non-fiber carbohydrates, fats and oils, proteins) in the diet. The latter impacts body substrate stores, the metabolic response to exercise and, potentially, athletic performance. These issues are discussed later in the chapter (see 'Feeds for attainment of nutritional goals').

Estimating energy requirements

In most parts of the world, both energy requirements and the energy content of feeds are evaluated as *digestible energy* (DE). In North America, the horse industry uses the kilocalorie (kcal) as the standard unit of energy, whereas use of the kilojoule (kJ) predominates in Europe and much of the rest of the world (4.184 kJ = 1 kcal). Digestible energy is expressed as kcal (or kJ) per gram of substance or Mcal (or MJ) per kg.

Several definitions of the energy requirements for maintenance have been used. These include: the daily food intake that maintains constant bodyweight and composition of a mature horse with zero energy retention at a defined level of activity; and the amount of DE required for zero bodyweight change plus normal activity of non-working horses. The work of Pagan & Hintz² indicated that maintenance energy requirements varied linearly with bodyweight and not with metabolic body size ($W^{0.75}$). Therefore, these researchers developed the following equation for prediction of maintenance energy requirements of horses weighing 200–600 kg:

$$\text{DE (Mcal/day)} = 1.4 + 0.03 W$$

where W is the weight (kg) of the horse. This equation overestimates the energy needs of large horses (<600 kg) and ponies and miniature horses, and this author uses $W^{0.75}$ when estimating the maintenance energy needs of these animals.

Several factors can influence maintenance energy requirements, including the age, breed, gender, and temperament of the horse, the level of activity, ambient conditions, and diet composition. Logically, therefore, 'book values' for maintenance energy needs based on these and other equations should only be considered a guide.

Similarly, there are no practical means for precise determination of the additional energy requirements of horses in exercise training. The intensity and duration of exercise, terrain, the weight of the rider and tack, the ability of the rider, the level of training of the horse, environmental conditions, and diet composi-

tion will all influence overall energy needs. Pagan & Hintz³ developed a prediction equation for the estimation of the DE required above maintenance using oxygen consumption ($\dot{V}O_2$) data collected from horses running on a level track:

$$\begin{aligned} \text{DE (kcal per kilogram of horse, rider, and tack)} \\ = \{[e^{(3.02+0.0065 Y)} - 13.92] \times 0.06\} / 0.57 \end{aligned}$$

where Y is the speed (meters per minute) and 0.57 accounts for the efficiency of utilization of DE. Although this equation may be useful for horses exercising on level ground and an even surface, the wider applicability of this approach is questionable given the need to measure running speed and the variation in terrain and footing conditions in which horses train and compete. Use of the equation developed by Pagan & Hintz³ underestimated by 30–35% the daily energy requirement of horses exercised on a treadmill for 60–75 min at 50% of maximum aerobic capacity ($\dot{V}O_{2\text{max}}$).^{4,5} In the future, the availability of systems for valid field measurements of oxygen consumption in horses undertaking different exercise tasks under a variety of conditions may facilitate development of more precise prediction equations. However, it is unlikely that such equations will account for all of the factors influencing energy requirements. As such, ongoing clinical assessment of bodyweight and condition will remain the most practical means for assessment of energy balance in horses.

In 2007 the NRC recommended that daily energy intake be increased 20%, 40%, 60%, and 100% above maintenance requirements for horses performing, respectively, light, moderate, and heavy and very heavy work. These recommendations were based on data from experimental studies, feeding surveys, and practical experience. Light work might include equitation and other forms of pleasure riding, while horses engaged in racing (Quarter horse, Standardbred, Thoroughbred, endurance) and elite three-day event would fall into the very heavy category (Table 5.3.1). Although these guidelines are clearly very general in nature, the results of field studies in working horses have provided evidence that the NRC recommendations are a useful guide for estimation of energy needs in horses in moderate and heavy work.^{6,7} For Standardbreds (mean bodyweight (bwt) of approximately 450 kg) and Thoroughbreds (mean bwt 500 kg) in race training, the NRC recommendations are 31.0 Mcal/day and 34.5 Mcal/day.¹ Consistent with these recommendations, Gallagher and co-workers estimated DE intakes of 28–31 Mcal/day for Standardbreds⁸ and 31–36 Mcal/day for Thoroughbreds⁶ in

Table 5.3.1 Estimated digestible energy (DE) requirements for a 500 kg horse at five different levels of activity

Activity*	Examples	DE requirement (Mcal/day)
Maintenance	Horse at pasture	15–18 (based on 30.3–36.3 kcal/kg bwt)
Light	Recreational riding Beginning of training programs Show horses (occasional)	20 [DE = (0.0333 × bwt) × 1.20]
Moderate	School horses Recreational riding Show horses (frequent) Polo	23.3 [DE = (0.0333 × bwt) × 1.40]
Heavy	Ranch work Polo Low–medium level eventing Race training (middle stages)	26.6 [DE = (0.0333 × bwt) × 1.20]
Very heavy	Racing (Quarter horse, Thoroughbred, Standardbred, endurance) Elite three-day eventing	34.5 [DE = (0.0363 × bwt) × 1.90]

*According to the 2007 NRC classification of activity level.
Mcal, megacalorie.
bwt, bodyweight.

North American race stables. Similar DE intakes were reported for Australian Standardbred and Thoroughbred race horses.⁷ Taylor et al⁹ reported that 420 kg Arabian horses undergoing treadmill exercise 3–4 days per week maintained bodyweight when fed 19–22 Mcal DE/day, equivalent to the NRC recommendation for horses in light work.

The effects of body condition and composition on athletic performance

In humans, there is a strong inverse relationship between body fat content and both sprint and endurance running performance. Although data from horses are limited, there also is evidence that body composition is an important determinant of athletic performance. Kearns et al¹⁰ used B-mode ultrasound of the rump area to calculate percentage of body fat and fat free mass (FFM) in 14 racing Standardbreds. Males had less fat mass (7.4% bwt) when compared to the females (9.9% fat). Interestingly, percentage fat mass was negatively correlated to race performance in the males but not the female horses of the study, a finding consistent with data in humans. However, for both genders, FFM was negatively correlated with race time (i.e. the higher the FFM, the shorter the race time). The authors concluded that while a low fat mass is beneficial to race performance, it is more

important to possess a larger FFM.¹⁰ A larger FFM is indicative of a greater muscle mass and, therefore, greater potential force development. Using similar methodology, this research group has demonstrated that maximum aerobic capacity ($\dot{V}O_{2\text{ max}}$) is also significantly related to FFM, independent of body mass.¹¹

Lawrence and colleagues¹² also used rump ultrasound to assess the relationship between body composition and endurance race performance. The average body fat content of the horses of this study (7.8%) was similar to that reported by Kearns et al¹⁰ for Standardbred race horses. Top finishers in a 150-mile race had a lower fat mass (by approximately 20 kg) when compared to horses that could not complete the event. Taken together, the results of these studies provide evidence that excess body fat is detrimental to sprint and endurance running performance.

On a more practical level, condition scoring is a useful clinical tool for the assessment of body fatness. Although several scoring systems have been used, the version developed by Henneke and colleagues at the Texas A & M University is the most widely used (Table 5.3.2).¹³ There are a few studies that have reported body condition score (BCS) in horses involved in different activities. In the aforementioned study by Lawrence and co-workers,¹² the mean BCS of horses in an endurance race was 4.67 (using the 1–9 scale).

Table 5.3.2 A body condition scoring system for horses

Condition	Score	Description
Poor	1	Severe emaciation; bone structure is prominent, including cervical and lumbar vertebrae
Very thin	2	Emaciation; bone structure is still prominent but cervical vertebrae barely visible
Thin	3	Neck thin; junction of neck, withers, and shoulder accentuated; pelvic structure remains prominent; transverse processes of lumbar vertebrae cannot be palpated
Moderately thin	4	Ribs prominent; spine still apparent but not individual vertebrae; neck, withers, and shoulder normal
Moderate	5	Ribs are not visible but easily palpated; neck blends smoothly into withers and shoulder; flat back
Moderately fleshy	6	Neck, shoulder, and withers appear more filled and rounded; back area may have slight depression (crease) along spine; tailhead and ribs with some flesh coverage
Fleshy	7	Fat deposited over withers and neck; crease along spine more visible; flesh over tailhead and ribs is prominent and soft
Fat	8	Thickened neck; well-defined crease along spine; ribs difficult to palpate; fat accumulation over rump
Extremely fat	9	Bulging fat in neck, withers, and shoulder; prominent crease down back; patchy fat over ribs

Adapted from reference¹³.

Gallagher et al reported that Standardbred and Thoroughbred horses in training had, respectively, mean BCS of 5.7 and 5.0.^{6,8}

Recent studies of endurance horses competing in the 100-mile Tevis Cup have provided evidence of a relationship between condition score and race performance.¹⁴ The mean BCS of horses that successfully completed the rides was 4.5, whereas horses that were eliminated for metabolic failure (e.g. colic, heat exhaustion, synchronous diaphragmatic flutter, or tying up) had a mean condition score of 2.9. Horses that were eliminated for non-metabolic reasons such as lameness and overtime had a mean condition score of 4.3. The researchers were careful to point out that their results may not apply to endurance competition as a whole, given the difficult nature of the Tevis Cup course. None the less, the implication from these studies is that there is an optimal level of 'fatness' for horses competing in endurance events and that training and feeding programs need to be adjusted accordingly. Endurance performance of horses with a low BCS (e.g. <3) may be limited by the supply of energy from fat reserves. In addition, a loss of lean tissue (muscle mass) may contribute to diminished performance in horses with poor body condition.

Although further studies are needed in horses of different types, as a general recommendation the BCS of most athletic horses should fall between 4 and 6.

The NRC recommendations should be used as an initial guide when making recommendations for DE intake in the face of changing work demands. Subsequently, however, frequent (e.g. weekly) assessment of bodyweight and/or BCS is necessary to determine the appropriateness of the feeding program; upward or downward adjustments in DE intake are often needed for maintenance of constant bodyweight. Trainers and/or owners should target a desired bodyweight or BCS, monitor these parameters on a weekly basis, and adjust energy intake accordingly.

Protein

The dietary protein requirement of the horse is a function of the amino acid needs, the amino acid composition of the dietary protein, and the digestibility of the protein. Horses have negligible ability to utilize non-protein nitrogen such that nitrogen needs must be provided in the form of protein or amino acids. Furthermore, it is thought that amino acids are not absorbed intact from the large intestine. Therefore, the horse's essential amino acid requirements must be met from protein sources that are digested in the small intestine. Whether, and to what extent, regular exercise increases the protein requirement is a controversial issue in both horse and human nutrition.

Theoretically, higher turnover of muscle proteins in association with the rigors of regular exercise (oxidation of amino acids during exercise, protein synthesis to support muscle hypertrophy and tissue repair after exercise) and sweat nitrogen losses should increase protein requirements. Consistent with this hypothesis, studies in humans have demonstrated negative nitrogen balance at the onset of endurance training programs. However, within 7–10 days of the start of training the rate of leucine oxidation is attenuated and nitrogen balance approaches equilibrium, indicating adaptation in protein metabolism.¹⁵ For the resistance-trained athlete, there also appears to be a homeostatic adaptation to the stress of exercise such that well-trained athletes require only marginally more protein than sedentary persons. For elite human endurance and strength athletes, a dietary protein intake that represents 14–15% of total energy intake (1.6–1.7 g protein/kg per day) has been recommended.¹⁵ Protein intake above these levels has not been shown to be beneficial to human athletic performance.

Protein requirements of horses have been expressed as a function of bodyweight or in relation to DE intake. For maintenance, 1.1–1.4 g crude protein (CP) per kg bodyweight is recommended or approximately 35–40 g CP/Mcal DE daily. The NRC¹ recommended that working horses in the light, moderate, heavy and very heavy categories receive, respectively, 1.4, 1.5, 1.7 and 2.0 g CP per kg bodyweight per day. It has been suggested that CP intakes greater than 2 g/kg bwt/day are detrimental to some forms of exercise because of the effects of excess dietary protein on heat production, acid–base balance, water requirements, and, potentially, respiratory health.^{16,17} Oxidation of the phosphorus and sulfur in protein adds to the acid load on the body. In this context, Graham-Thiers and colleagues¹⁶ evaluated the effects of a restricted protein diet (7.5% CP with added lysine and threonine) on acid–base balance in horses in moderate work. When compared to a 14.5% CP diet, protein restriction resulted in a slight increase in resting blood pH and mitigation of exercise-associated acidemia during repeated sprints. These effects may provide a performance advantage during exercise, although the actual effect of dietary protein level on exercise performance has not been determined.

There is evidence that dietary protein level alters urea metabolism in horses. In one study, horses consuming 1741 g CP per day (>3 g/kg bwt) excreted more urea in sweat and had higher plasma urea when compared to horses consuming 836 g CP per day¹⁸

and it has been estimated that a change in dietary CP from 10% to 15% would increase water requirement by approximately 5% because of an obligate increase in urine production for clearance of endogenous urea loads.^{17,19} Moreover, the higher urinary urea load could adversely affect the respiratory health of confined horses because urea is converted to ammonia, a known respiratory irritant.

Further research is needed regarding the protein requirements of athletic horses. Currently, a 10–12% CP diet is recommended for mature athletic horses. However, the recent studies by Graham-Thiers and co-workers¹⁶ suggest that there may be advantages to a lower protein diet (8–10% CP), providing good-quality protein is fed.

Minerals and vitamins

Based on their daily requirements, minerals are usually classified as macrominerals (e.g. calcium, phosphorus, sodium, potassium, and chloride) or trace elements (e.g. selenium, iron, zinc, copper). These nutrients are important in a large array of body functions. Several minerals and trace elements, such as magnesium, iron, zinc, and copper, act as enzyme activators in glycolysis and oxidative phosphorylation. Minerals (electrolytes) are also critical to nerve and muscle function.

The classification of vitamins is based on their relative solubility; fat-soluble vitamins (A, D, E, and K) are more soluble in organic solvents, whereas the B vitamins and vitamin C are more soluble in water. Most vitamins participate in processes related to muscle contraction and energy expenditure. Vitamins of the B complex group (e.g. thiamine, riboflavin, vitamin B₆, niacin, biotin, and pantothenic acid) act as cofactors for enzymes regulating glycolysis, the citric acid cycle, oxidative phosphorylation, β -oxidation of fatty acids, and amino acid catabolism. Folic acid and vitamin B₁₂ are needed for heme synthesis. The antioxidant vitamins (mainly vitamins C and E) participate in a buffer system against free radicals.

Many trainers, owners, and veterinarians perceive that optimal mineral and vitamin nutrition is critical to successful athletic performance. One impression is that the rigors of training and competition promote excessive losses of these nutrients because of increased excretion or catabolism and, as a result, fortification of the diet is required to cover these losses. Moreover, there is often the opinion that increased consumption of various minerals and vitamins will improve physi-

Table 5.3.3 Estimated daily mineral needs of a 500 kg^a horse at maintenance and in moderate-to-heavy exercise training

Mineral	Maintenance	Exercise
Sodium (g)	10	20–40 ^b
Potassium (g)	25	30–60 ^b
Chloride (g)	40	50–100 ^b
Calcium (g)	20	35–40
Phosphorus (g)	15	21–29
Magnesium (g)	7.5	11–15
Iron (mg)	400	450–500
Copper (mg)	100	115–125
Manganese (mg)	400	450–500
Zinc (mg)	400	450–500
Selenium (mg)	1.0	1.1–1.25
Iodine (mg)	3.5	4.0–4.4
Cobalt (mg)	0.5	0.6

^aAssumes a dry matter intake of approximately 2.0%, 2.25%, and 2.5% of bodyweight for maintenance, moderate exercise, and heavy exercise, respectively.

^bThe sodium, potassium, and chloride requirements of working horses are, in part, dependent on the extent of sweat fluid losses. The upper end of these values is recommended for horses in intense training in warm climates and during the summer months.

Adapted from references^{1,40}.

cal performance and health. These perceptions, together with the aggressive marketing of supplements containing minerals and vitamins, appear to provide impetus for the extensive use of nutritional supplements in horses.

Macrominerals

Recommendations for macromineral (and trace mineral) intake by mature athletic horses are presented in Table 5.3.3. The requirements for dietary calcium (Ca) and phosphorus (P) are higher in athletic horses in comparison to their sedentary counterparts. Diets that contain inadequate levels of these nutrients or an imbalanced Ca:P ratio (i.e. Ca:P ratio < 1; high dietary P) may compromise bone integrity and predispose to injury during training and competition. Additional Ca and P are required for deposition in bone during modeling and remodeling, processes that are upregulated by the loading imposed on the bones of the limbs during exercise. It is also possible that calcium losses in sweat increase dietary requirements.

However, assuming a calcium concentration in sweat of 100–120 mg per liter and daily sweat losses of 10 liters, these losses may be no more than 1–2 g per day.

With respect to Ca and P requirements, the guidelines furnished by the NRC¹ assumed that if the nutrient:calorie ratio of the ration was maintained, the increase in feed intake needed to meet DE requirements for work would also supply the calcium required to replace sweat losses and support bone formation. However, the calcium needs of young horses in training (<2 years) may be higher in comparison to mature athletic horses because of greater Ca turnover in bone.^{20,21} Nolan and colleagues²¹ reported that the training-associated increase in bone density in young Quarter Horses was enhanced by the consumption of diets that provided 150–170% of the NRC (1989) recommendations for Ca. In line with these findings, it has recently been suggested that diets for young horses in training should contain at least 0.4–0.45% Ca (versus the NRC recommendation of 0.34% Ca).²⁰ Higher levels of dietary calcium may also be beneficial in older horses. In one study, the increase in the mineral content of the metacarpus, assessed by radiographic photometry, in response to 12 weeks of treadmill training was greater in horses receiving a diet containing 0.7% Ca when compared to the current recommendation of 0.34%.²²

Close attention should be paid to Ca:P balance in horse rations; the Ca:P ratio should be at least 1:1. It is not uncommon for athletic horses to be fed a diet with an improper Ca:P balance. This is particularly true for horses fed high-cereal grain (not fortified with minerals), low-roughage diets because most cereals are low in Ca and high in P. Although most grass hays contain moderate Ca content, the quantities fed may be insufficient to balance the low Ca content of the grain, resulting in an inverted Ca:P ratio (<1). This type of ration can result in bone demineralization and pathology.²³ This problem can be averted by provision of a supplemental source of Ca or by including some legume hay in the diet. On the other hand, the Ca:P ratio of diets containing large amounts of legume hay can be greater than 3–5:1. However, such diets appear to be well tolerated by mature horses.

The NRC guidelines for Ca and P nutrition are based on the assumption that the efficiency of absorption for Ca and P is 50% and 35% respectively. However, several factors can influence the efficiency of absorption of these nutrients, including the Ca:P ratio, the form of Ca or P, the level of intake, and the presence of inhibitors such as phytate and oxalate.

Table 5.3.4 The composition of selected calcium and phosphorus supplements

Supplement	Calcium (%)	Phosphorus (%)	Magnesium (%)	Sulfur (%)
Calcium carbonate	39	–	0.05	–
Limestone	34	0.02	2.1	0.04
Oyster shells, ground	38	–	0.3	–
Dicalcium phosphate	22	19	–	–
Monosodium phosphate	–	22	–	–
Magnesium oxide	0–3	–	51–59	–

Grains and cereal hays are high in phytates, while some tropical grasses are high in oxalates such that the efficiency of digestion and absorption of Ca and P in these feedstuffs is low. On the other hand, the efficiency of absorption of inorganic source Ca and P (e.g. calcium carbonate, dicalcium phosphate) may approach 70%. Due to the high variability in the availability of Ca and P from organic sources, most commercial horse feeds use inorganic sources of Ca and P to ensure adequate dietary levels of these nutrients. Similarly, inorganic forms of Ca and P should be added to 'homemade' rations for athletic horses (Table 5.3.4).

Scant data are available regarding the magnesium (Mg) requirements of athletic horses. In one study of endurance horses, serum magnesium concentrations were unchanged after an 80 km race.²⁴ Muscle (intracellular) magnesium content was unchanged in horses after intense trotting over 2600 m.²⁵ However, athletic horses may have slightly increased requirements for dietary magnesium to cover sweat losses (equine sweat contains 100–120 mg magnesium per liter). The NRC¹ recommends 0.1–0.12% dietary magnesium for maintenance or approximately 11–15 g/day based on a daily dry matter (DM) intake of 11–12 kg. As the magnesium content of feeds commonly consumed by horses is 0.1–0.3%, typical daily intake by athletic horses will be much greater than 11–15 g/day and should cover daily losses associated with sweating.

The dietary requirements for sodium, potassium, and chloride are increased by physical activity because of the substantial sweat loss of these ions. The extent of these losses will depend on the intensity and duration of exercise, the training and heat acclimation status of the horse, and the ambient conditions. A more detailed discussion of the effects of exercise on fluid and ion balance, strategies for electrolyte replace-

ment, and daily electrolyte requirements can be found elsewhere in this book.

Trace minerals

Few data exist regarding the effects of regular physical activity in horses on trace mineral requirements. With the exception of selenium, clinical signs consistent with a trace mineral deficiency are rare in horses fed typical diets. None the less, several of the trace minerals are routinely supplemented to working horses. Most notably, iron, often in combination with copper, zinc, and some of the vitamins, is widely given to athletic horses because of the perception that the provision of these nutrients will enhance erythropoiesis and oxygen-carrying capacity. In reality, there is no evidence that this practice results in altered red cell number or hemoglobin concentration, and iron deficiency anemia is a rare diagnosis in horses. Daily Fe losses of horses in training have not been reported. However, sweat contains a small amount of iron and other trace minerals such as zinc. Therefore, there is some rationale for increased provision of dietary Fe in working horses. Practically, however, common horse feedstuffs provide iron well in excess of requirements. The NRC maintenance requirement for iron is 40 mg/kg of diet (DM basis) or approximately 0.8 mg/kg bwt/day (400 mg/day for a 500 kg horse).¹ Forages usually contain 100 mg Fe per kg DM and most grains more than 50 mg per kg DM. Thus, a diet of 6 kg of hay and 5 kg of grain concentrate for a 500 kg working horse would provide approximately 850 mg Fe.

Selenium is a functional component of the intracellular enzyme glutathione peroxidase and, in concert with vitamin E, is important in the defense of cells against oxidant stress. Selenium deficiency is associated with development of nutritional myodegenera-

tion. Selenium is also essential for the development of acquired immunity. Whether regular physical activity increases the Se requirements of horses is not known. However, single bouts of exercise²⁶ have been reported to increase erythrocyte glutathione peroxidase activity. This increase in enzyme activity may be a reflection of an increase in demand on this antioxidant system and, perhaps, an indication that working horses have higher Se needs relative to idle horses. The NRC¹ concluded that the Se requirement for mature idle horses was 0.1 ppm on a dry matter basis (0.1 mg/kg diet, assuming a DM intake of about 2% of bodyweight per day). Yet, many nutritionists now favor higher dietary Se for idle and working horses, in the range of 0.2–0.3 ppm, to account for variation in the efficiency of selenium digestibility and utilization and increased work demands.

Vitamins

There is very limited information regarding the vitamin requirements of horses and even fewer data concerning the effects of habitual exercise on these requirements. Microbes in the large intestine synthesize the B vitamins and absorption from the large intestine has been demonstrated for several of the B vitamins.²⁷ In addition, an ample supply of the B-complex vitamins, with the exception of vitamin B₁₂, is provided by good-quality forage. Therefore, a combination of dietary intake and microbial synthesis in the hindgut should meet the B vitamin requirements of horses.

In recent years, there has been considerable interest regarding the role of vitamins C and E (and other antioxidant nutrients such as β -carotene and lipoic acid) in the mitigation of oxidative stress and lipid peroxidation in horses during exertion. Vitamins E (α -tocopherol) and C, along with glutathione, are important non-enzymatic antioxidants. Vitamin E is a lipid-soluble scavenger of reactive oxygen species (ROS) that is located in cell membranes, while vitamin C is located in the aqueous phase of cells where it scavenges free radicals and recycles vitamin E to its active form.²⁸

Exercise causes a dramatic increase in oxidative metabolism and, therefore, the production of ROS. During prolonged and/or strenuous exercise, the extent of ROS production may overwhelm antioxidant systems such that the susceptibility of cells and tissues to damage by ROS (lipid peroxidation) is enhanced, particularly in animals with inadequate endogenous stores of the antioxidant nutrients. Several research

groups have investigated the relationship between exercise and plasma markers of oxidative stress in horses. For example, Hargreaves et al²⁶ reported an association between muscle cell 'leakage' (exercise-associated increases in the activities of creatine kinase (CK) and aspartate aminotransferase (AST)) and indices of antioxidant status in horses during 80 and 160 km endurance races. Specifically, plasma vitamin C and erythrocyte glutathione concentrations decreased during the races, while erythrocyte glutathione peroxidase was increased. On the other hand, Marlin and colleagues²⁹ reported no change in plasma vitamin C after a 140 km race, but significant decreases after 16 h of recovery. In both studies, no changes in plasma α -tocopherol concentrations were detected. However, Marlin et al²⁹ demonstrated a negative correlation between pre-race plasma α -tocopherol and total red cell glutathione and end-competition total barbituric acid reactive substances (TBARS), and suggested that horses with low antioxidant reserves may be more prone to lipid peroxidation from ROS during endurance exercise.

The NRC¹ recommended a dietary vitamin E concentration of 50–80 IU/kg DM for all classes of horse. Whether or not higher amounts of dietary vitamin E are beneficial in athletic horses is unresolved. Siciliano et al³⁰ demonstrated a decrease in plasma and muscle α -tocopherol concentrations in working horses fed a diet supplemented with 80 IU α -tocopherol per kg DM, whereas plasma concentrations were maintained when horses were supplemented with 300 IU/kg DM. Similarly, plasma concentrations of α -tocopherol were significantly higher in hard-working polo ponies fed a diet providing 300% of the current NRC recommendation for vitamin E (240 IU/kg versus 80 IU/kg diet).³¹ In both studies, however, there was no association between dietary vitamin E and the serum activities of CK and AST in response to exercise. Further research is needed to better define the vitamin E requirements for different classes of athletic horse. In the meantime, it is recommended that athletic horses be fed a diet providing at least 100 IU vitamin E per kg DM. Intensely working horses (e.g. race and endurance horses) may benefit from higher levels of vitamin E (200–250 IU/kg diet). Most equine feedstuffs contain less than 50 IU/kg DM. Therefore, supplemental vitamin E must be added to horse rations to meet these suggested requirements.

As horses have L-gluconolactone oxidase, the enzyme required for the synthesis of vitamin C (ascorbic acid) from glucose, there is no dietary requirement for this nutrient. However, the demand for ascorbic

acid may be increased by regular exercise because it is thought to spare tissue vitamin E by reducing the tocopheroxyl radical and restoring the radical scavenging activity of vitamin E.³² Although there have been several studies on the effects of ascorbic acid metabolism and supplementation in athletic horses,^{29,31,33–35} no consensus has emerged regarding a dietary requirement for horses under performance stress. Dietary supplementation with ascorbic acid (5–10 g/day) does result in higher serum ascorbate concentrations relative to non-supplemented horses.^{29,31} However, the effects of these higher ascorbate concentrations remain unclear. In two equine studies, there was no effect of ascorbic acid supplementation on exercise-induced increases in serum CK activity.^{31,33} One concern with high-level ascorbic acid supplementation is the potential for suppression of endogenous synthesis.

Water

The NRC guidelines recommend 4–6 liters of water per 100 kg of bwt per day.¹ However, diet, ambient conditions, and level of activity will modify these needs. Horses maintained on an all-forage diet will consume approximately 3.6 liters of water per kg of DM intake, whereas the water-to-feed ratio for horses fed a typical hay and grain diet is about 3:1.¹ Water intake by horses living in warm climates (>25–30°C daytime temperature) may be increased by 15–30%, whereas heavy work in warm to hot ambient conditions may increase the need for water by as much as 200–300% due to large sweat fluid losses.

Feeds for attainment of nutritional goals

For horses in training, a combination of forage and energy concentrate is generally required to achieve nutritional goals. In some situations, a vitamin–mineral supplement is added to the ration. Several guiding nutritional principles should be used in the development of a feeding program for an athletic horse. In general, the main considerations in ration formulation are:

- provision of adequate fiber (roughage) to maintain normal gut and digestive function (and perhaps limit the development of behavioral disturbances)

- targeting an overall energy density that will allow energy requirements to be met at typical fed intakes
- supplying sufficient hydrolyzable carbohydrate to maintain muscle glycogen concentrations
- provision of the optimal amounts and balance of the other essential nutrients (i.e. protein, minerals, vitamins)
- the inclusion of only the highest-quality feedstuffs.

The four main sources of energy in horse rations are:

1. fermentable carbohydrates (components of dietary fiber or roughage, including hemicellulose) that cannot be digested by mammalian enzymes but can be fermented by micro-organisms, primarily in the hindgut, i.e. cecum and large colon
2. hydrolyzable carbohydrates (simple sugars and starch) that are digested by mammalian enzymes in the small intestine, yielding hexoses
3. oils and fats
4. protein (not primarily fed as an energy source because metabolism of amino acids to usable energy is inefficient).

Knowledge of the horse's digestive physiology and the impact of diet composition and meal size on the efficiency of digestive processes are important in the selection of an appropriate ration and feeding strategy. The horse evolved as a grazing animal and its gastrointestinal tract, with a well-developed cecum and large colon, is highly adapted to the utilization of fiber-rich feeds that are consumed on an almost continuous basis.³⁶ The hindgut (cecum and colon) comprises approximately 64% of the total (empty) volume of the gastrointestinal tract, whereas the stomach (7%) and small intestine (25%) have a relatively small capacity. Similar to other mammalian species, the small intestine is the major site of digestion of protein, fat, and hydrolyzable carbohydrates. However, as further discussed below, the horse has a limited ability to digest and absorb hydrolyzable carbohydrates (particularly starch) in the small intestine. Large concentrate meals may overwhelm the digestive capacity of the small intestine and promote the flow of undigested hydrolyzable carbohydrate to the large intestine. This not only reduces the efficiency of feed utilization but also increases the risk for digestive disturbances associated with excessive and uncontrolled fermentation of the undigested hydrolyzable carbohydrate in the large intestine.

Roughage/dietary fiber

From the preceding discussion, it is clear that forage (roughage) should always be the foundation of an equine ration. Although a requirement for dietary fiber has not been established in horses, some long-stem roughage is important for maintenance of normal hindgut function and thus for normal digestion. There also is evidence that diets low in long-stem fiber favor development of certain stereotypies.^{37–39} Therefore, adequate dietary roughage may be important for prevention of some undesirable behavioral traits, particularly in horses kept in confinement. Some trainers prefer to feed low-roughage diets because such rations may reduce the weight of ingesta in the intestinal tract ('dead weight'), thereby providing an energetic advantage during some forms of exercise. The possible benefits of this practice should be weighed against the increased risks of gastrointestinal dysfunction (e.g. colic, gastric ulcers) and behavioral abnormalities when horses are fed low-roughage diets.

Meyer has suggested that performance horses be fed at least 0.5 kg of roughage per 100 kg bwt (0.5% of bwt).⁴⁰ However, this author recommends *at least* 1.0 kg forage per 100 kg (i.e. 5.0 kg for a 500 kg horse). One survey of Australian race horses indicated that forage intake is often less than this minimum recommendation. In that study, mean daily forage intake was 3.3 kg and 4.1 kg for Thoroughbreds and Standardbreds, respectively.⁷

Pastures and different forms of conserved forage (i.e. hay, chaff, hay cubes, haylage) are the primary

source of roughage in horse rations. Although several factors can affect the nutrient value of conserved forages, the most important is the stage of maturity at the time of harvest (Fig. 5.3.1). The energy content, digestibility, and palatability of forage all decrease with increasing maturity. Therefore, forages harvested at an early stage of plant maturity should be fed to working horses to maximize nutritional value and intake of the offered quantities (Table 5.3.5). An exception to this recommendation is the feeding of a very high-quality alfalfa, which may not contain adequate fiber (specifically, acid detergent fiber (ADF)) for normal hindgut function. Figure 5.3.2 illustrates how the energy content of the forage affects the relative proportions of forage and energy concentrates required to meet the energy needs of an athletic horse.

The relative use of grass, legume, or cereal (usually oats) hays and the different forms of preserved forage made from these species often depends on availability and personal preference. Legume hays such as alfalfa usually have higher energy, protein, and mineral (particularly calcium) content when compared to the grass species. These differences will influence the type and quantity of energy concentrate to be fed with hay. For example, the total diet should provide 10–12% crude protein. Therefore, when feeding alfalfa the protein content of the concentrate can be lower than when grass hay is fed. In addition, a higher roughage-to-concentrate ratio is possible when high-quality (energy) forage is fed. However, even within a plant species the nutrient composition of preserved forage can vary greatly depending on growing conditions,

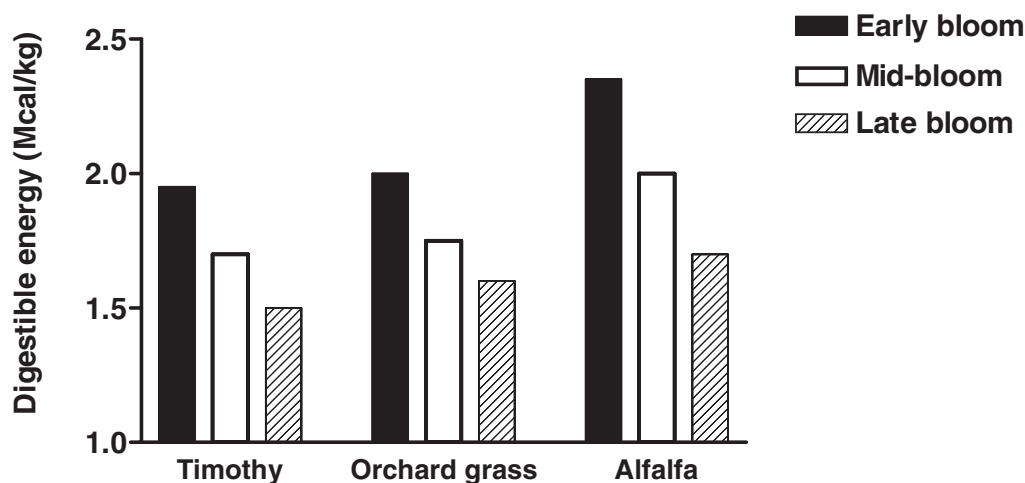
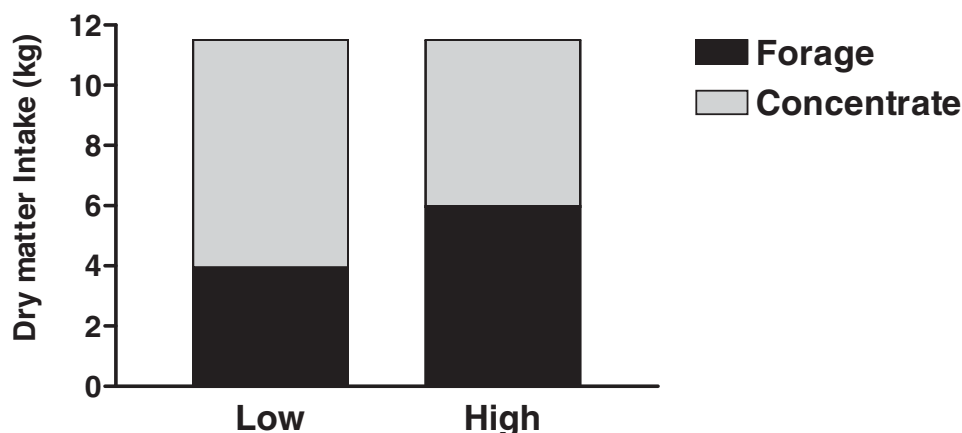


Fig. 5.3.1

The effects of maturity at the time of harvest on estimated digestible energy content of timothy, orchard grass, and alfalfa hay.

**Fig. 5.3.2**

The effects of forage energy content on the proportions of hay and energy concentrate required to meet the energy requirements of a 500 kg horse with daily digestible energy (DE) needs of approximately 30 Mcal/day. This example assumes a daily dry matter (DM) intake of 11.5 kg or approximately 2.2% of bodyweight. Low = hay with DE content of 1.5 Mcal/kg DM; high = hay with DE 2.0 Mcal/kg. The DE content of the energy concentrate is 3.3 Mcal/kg. The diet proportions in these two scenarios are: low (4 kg hay and 7.5 kg concentrate or a ratio of 35:65); high (6 kg of hay and 5.5 kg of concentrate or a ratio of 52:48).

Table 5.3.5 Nutrient composition of some feedstuffs used in horse rations

Feed	Dry matter %	DE (Mcal/kg DM)	CP %	Acid detergent fiber %	NSC %	Fat %	Ca %	P %
Alfalfa hay, early bloom	90	2.5	20	32	22	—	1.4	0.3
Alfalfa hay, full bloom	91	2.2	17	39	21	—	1.2	0.25
Timothy hay, early bloom	90	1.9	10	35	17	—	0.45	0.26
Timothy hay, full bloom	90	1.7	7.5	40	17	—	0.3	0.2
Rice hulls	92	0.5	3	72	7	—	0.12	0.07
Rice bran	91	2.9	14	20	14–15	20–22	0.1	1.5–1.7
Oats	90	3.2	10–13	16	49.5	4.5	0.1	0.35
Barley grain	89	3.7	13	8	62	1.7	0.05	0.34
Corn grain	88	3.85	8–10	4	66	3.7	0.05	0.3
Beet pulp	91	2.6	10	27.5	38	—	0.7	0.1
Soy hulls	92	2.0	11–13	46–54	—	—	0.4–0.7	0.15–0.2
Molasses	78	3.4	2–6	0	77	—	0.15	0.03
Wheat bran	89	3.3	16–17	13–15	15–17	—	0.14	1.27

DE, digestible energy; CP, crude protein; NSC, non-structural carbohydrate; Ca, calcium; P, phosphorus.

All nutrients expressed as a percentage in feed dry matter.

Primary source of data is 2007 NRC.

plant maturity, and harvesting methods. Thus, proximate analysis (including crude protein, neutral detergent fiber, and macrominerals) should be performed when practical to best guide ration balancing and the selection of an appropriate energy concentrate.

Preserved forages should also be free of contaminants such as molds. Hay that is baled at high moisture content is likely to heat and become heavily contaminated with molds that can exacerbate chronic airway diseases such as recurrent airway obstruction and inflammatory airway disease. In areas with high rainfall during the growing and hay-making season (e.g. the UK), the feeding of ensiled hay or 'haylage' (50–60% DM) is sometimes practiced. Haylage is a suitable alternative to hay providing that it is stored correctly and fed in sufficient quantity to ensure adequate fiber intake. One concern with haylage is the potential for clostridial growth and production of botulinum toxin.⁴¹ Haylage should be examined carefully for the presence of mold and other contaminants before feeding and sealed haylage bales should be fed within a few days of opening. Hay cubes and pellets, 'complete feeds', or the feeding of forage that has been thoroughly soaked in water can also aid in minimizing exposure to dusts and molds when managing chronic airway disease.

Daily feed intakes in mature horses (on an as-fed basis) range between 1.5% and 3.0% of bodyweight, although a more typical intake for a performance horse is between 2.0% and 2.5% (i.e. 10–12.5 kg for a 500 kg horse). Accordingly, the energy requirements of horses performing light athletic activities (e.g. pleasure riding 2–3 times per week) can, in most instances, be met by forage alone. Other essential nutrients may be provided in the form of a vitamin–mineral or vitamin–mineral–protein supplement. On the other hand, horses in moderate and intense training are unable to meet energy needs if forage is the only energy source. Therefore, energy concentrates must be fed to increase the caloric density of the diet and ensure that energy requirements are met within the confines of a realistic daily dry matter intake.

Energy concentrates

Cereal grains

Traditionally, cereal grains such as oats, corn, and barley (alone or in combination) have been a source of energy in rations for athletic horses. Starch, a hydrolyzable carbohydrate, is the primary component

of cereal grains. Oats are approximately 47–50% starch while the starch content of corn and barley is between 60% and 66%. Digestion of starch in the small intestine yields glucose, the substrate for liver and muscle glycogen synthesis. As muscle glycogen is a primary fuel during exercise, the provision of some hydrolyzable carbohydrate (starch and/or sugar) in the diet of an athletic horse is important for replenishment of glycogen reserves. However, there is evidence that the horse has a limited capacity to digest and absorb starch (and perhaps other simple carbohydrates) from the small intestine. Low production and secretion of pancreatic amylase^{42,43} and a limited capacity for mucosal monosaccharide transport⁴⁴ are factors that may contribute to this apparent constraint in small intestinal carbohydrate digestion. Regardless of the mechanism, with the ingestion of large grain meals a substantial proportion of the ingested starch may escape hydrolysis in the small intestine with a resultant delivery of this substrate to the hindgut. Rapid fermentation of starch in the hindgut by lactate-producing bacteria can result in lactate accumulation, excess gas production, cecal and colonic acidosis, and increased risk of intestinal disturbances.^{45,46} Epidemiological studies have identified the level of grain feeding as a risk factor for colic. Tinker et al⁴⁷ reported odds ratios of 4.8 and 6.3 (relative to no episode of colic) for horses fed, respectively, 2.5 kg/day and more than 5.0 kg/day of concentrate. Similarly, Hudson and colleagues⁴⁸ reported that recent (within the previous 2 weeks) changes in the type of grain or concentrate fed or feeding more than 2.7 kg of oats per day was associated with increased risk for an episode of colic. High-starch (grain) diets have also been implicated in the pathogenesis of some forms of chronic exertional rhabdomyolysis.⁴⁹

While it is necessary to feed some hydrolyzable carbohydrate to athletic horses to ensure an adequate supply of substrate for glycogen replenishment, several strategies can be employed to mitigate the risk of digestive disturbances attributable to heavy grain (starch) feeding. First, it is advisable to limit the size of individual grain-based meals to avoid 'starch bypass' to the large intestine. Second, only cereal grains with high pre-cecal starch digestibility should be included in energy concentrates for horses. Third, energy concentrates for athletic horses should make more use of non-starch carbohydrates (e.g. sugar beet pulp) and vegetable fats. Inclusion of these alternative energy sources facilitates a reduction in the level of starch feeding without compromising the caloric density of the ration.

A suggested upper limit of starch intake in a single meal is between 2 and 4 g starch per kg bodyweight (0.2–0.4% of bwt per feeding),^{43,50} although one nutritionist has suggested that 2 g/kg per meal is the safe upper limit.⁴⁶ Thus, if a concentrate feed contains 50% starch (e.g. plain oats) the maximum recommended amount of concentrate per feeding is approximately 2 kg for a 500 kg horse. Pre-cecal starch digestibility varies with the type of grain and the nature of any mechanical or thermal processing. For example, whereas oat starch (at up to 3 g/kg per meal) has a pre-cecal digestibility of greater than 90%, approximately 35% of an equivalent dose of cornstarch reaches the cecum undigested.⁴⁶ Similarly, the pre-cecal digestibility of unprocessed barley is substantially lower when compared to oats. However, heat treatments such as micronization, extrusion, and steam flaking significantly improve the pre-cecal starch digestibility of barley and corn. Overall, oats appear to be the safest source of starch for horses, although barley and corn are acceptable if they are subjected to some form of heat treatment.

Fats and oils

The addition of fat to horse rations is now commonplace. Although animal fat (tallow or lard) has been fed to horses, palatability and digestibility^{51,52} are inferior when compared to vegetable oils. Therefore, most commercial fat-supplemented concentrates for horses contain a vegetable oil such as soy, corn, or canola. Other oils that may be used in equine rations include peanut, safflower, coconut, linseed, or flaxseed. Vegetable oils are highly unsaturated and contain approximately three times as much DE as oats and 2.5 times as much as corn. Other sources of fat used in horse rations include stabilized rice bran (18–22% fat), flaxseed meal (40% fat), and copra meal (8–9% fat). Vegetable oils are both highly digestible (90–100%)⁵³ and palatable,⁵² although there can be slight variation in the acceptance of the different oils. For example, Holland et al⁵² demonstrated that corn oil is preferred over soy, peanut, and cottonseed oils. Rice bran (as a powder or an extruded pellet) is also well accepted by horses and is commonly fed at a rate of 0.5–2.5 kg per day to mature horses, providing 90–500 g fat per day. Rice bran is rich in phosphorus and has an inverted Ca:P ratio, but many commercial rice bran products contain added calcium (e.g. calcium carbonate) to correct this imbalance. Alternatively, a mineral supplement can be added to the ration to ensure an appro-

priate Ca:P ratio (at least 1:1) in rations containing rice bran.

Fat is often added to the diet to increase the energy density of the ration, which can offer an advantage when dry matter intake limits provision of adequate energy to maintain condition ('hard keeper' horses). Alternatively, substitution of fat for a portion of the grain in an energy concentrate allows for a decrease in hydrolyzable carbohydrate intake. This strategy is advocated for horses with some forms of chronic exertional rhabdomyolysis (see below).

Fat is also added to the diet of athletic horses because of the reputed benefits of fat-supplemented diets on exercise performance, specifically that performance is improved by *fat adaptation*, a set of physiologic responses to the consumption of a high-fat diet during training that confer advantages to the horse during exercise.⁵⁴ As discussed in Chapter 5.1, there is evidence that a fat-supplemented diet is associated with an increased capacity for fat oxidation during exertion, as shown by a lower respiratory exchange ratio during low- and moderate-intensity exercise.^{55,56} However, this effect of fat supplementation on fuel selection is not evident at higher workloads, reflecting the high dependence of horses on carbohydrate metabolism during moderate- and high-intensity exercise.⁵⁵ None the less, the increase in fat oxidation and decrease in carbohydrate oxidation (carbohydrate-sparing) evident during low-intensity exercise in fat-supplemented horses should be beneficial during endurance exercise, although the actual effect of fat supplementation on endurance performance has not been reported.

It should also be noted that the level of fat supplementation used in the aforementioned research studies (approximately 20–25% of DE from fat)^{55,56} is considerably higher than that commonly practiced by horse owners and trainers (4–15% of DE from fat). Whether these lower levels of fat supplementation confer similar metabolic advantages during exercise is unknown.

The ideal amount of dietary fat for horses has not been determined, nor is there much information regarding the effects of fatty acid chain length or degree of saturation on metabolism and health in horses. Kronfeld⁵³ has stated that horses can safely consume diets containing up to 15–20% vegetable oil by weight (on a total diet basis), providing the horse is adapted to this level of fat inclusion over a 2–3-week period. However, one study demonstrated a decrease in total tract fiber digestibility in horses fed a 14% soy oil diet.⁵⁷ Commercially, the level of fat or oil added to a concentrate is often limited by manufacturing con-

straints (e.g. poor pellet quality when large amounts of oil are included in pelleted feeds; greasy appearance of concentrate mixes). Therefore, fat-supplemented concentrates designed for performance horses usually contain between 5% and 14% fat (as fed), providing between 8% and 30% of the DE. However, as these concentrates are fed with forage, the amount of fat on a total diet basis is much lower (approximately 3–8% fat or 4–15% of the DE from fat assuming a 50% concentrate, 50% forage diet). These levels of fat supplementation carry minimal risk of negative associative effects such as a decrease in fiber digestibility.

Many horse owners and trainers add vegetable oil to existing rations. A suggested upper limit of oil supplementation is 100 g per 100 kg bwt per day. For reference, one standard measuring cup contains 250 ml (8 fluid ounces) of oil (~200 g) and provides approximately 1.67 Mcal (6.7 MJ) of DE. For a 500 kg horse in moderate work (daily DE needs of 26 Mcal), the inclusion of 500 g of oil per day will provide about 16% of the DE requirements. There should be a gradual introduction to oil feeding to avoid digestive disturbances (loose and oily feces). Initially, $\frac{1}{4}$ – $\frac{1}{2}$ cup of oil/day can be added to the ration. Over a 2–3-week period, the amount of added oil can be increased to 2–2 $\frac{1}{2}$ cups/day, divided into at least 2–3 feedings.

One concern with the addition of vegetable oils (or rice bran) to an existing diet is the potential for nutrient imbalances. Commercial energy concentrates with added fat are fortified to maintain appropriate nutrient-to-calorie ratios and are designed to complement the forage source. On the other hand, the on-farm addition of a substantial amount of oil (e.g. 400–500 g per day) to an existing ration may result in an unbalanced diet. Consultation with a nutritionist is recommended in these situations. Supplementation with vitamin E (100–200 IU per 100 g of added oil) is recommended for prophylaxis against oxidant stress when oil is directly added to the ration. This practice may not be necessary when rice bran is the source of added fat as it contains substantial quantities of vitamin E and other natural antioxidants.

Non-starch carbohydrates

There are two main types of non-starch polysaccharide used in equine rations: simple sugars; and highly digestible sources of fiber (so-called 'fermentable fibers'), particularly sugar beet pulp (SBP), soya hulls and, to a lesser extent, citrus pulp. Simple sugars in the form of molasses (a mixture of glucose, sucrose, and fructose) are often added to grain mixes at 6–8%

by weight. In general, these sugars are well utilized by the horse, as shown by pronounced glycemic responses following the administration of glucose, sucrose, or fructose at dosages of 1.0–2.0 g/kg bwt.⁵⁸

SBP contains major fractions of highly digestible fiber fractions, including pectins, arabinans, and galactans, which are readily fermented by equine hindgut microflora.⁵⁹ Digestibility studies have demonstrated that, in contrast to the fibers in traditional roughage sources, the fibers in SBP and soya hulls are extensively degraded within the time that such a feed-stuff would be resident in the gut.⁶⁰ This high digestibility accounts for the higher energy value of these feedstuffs when compared to hay. It has also been demonstrated that the addition of 30% SBP to the ration increases the digestibility of the hay portion of the diet.⁶⁰ SBP is available in two forms, molassed (i.e. molasses is added to the beet shreds, generally at the 5% level) or non-molassed, and can be fed alone or as a component of a concentrate mix. When fed alone, it is recommended to soak the beet shreds in water for 3–4 hours before feeding. Soya hulls are generally included in pelleted feeds.

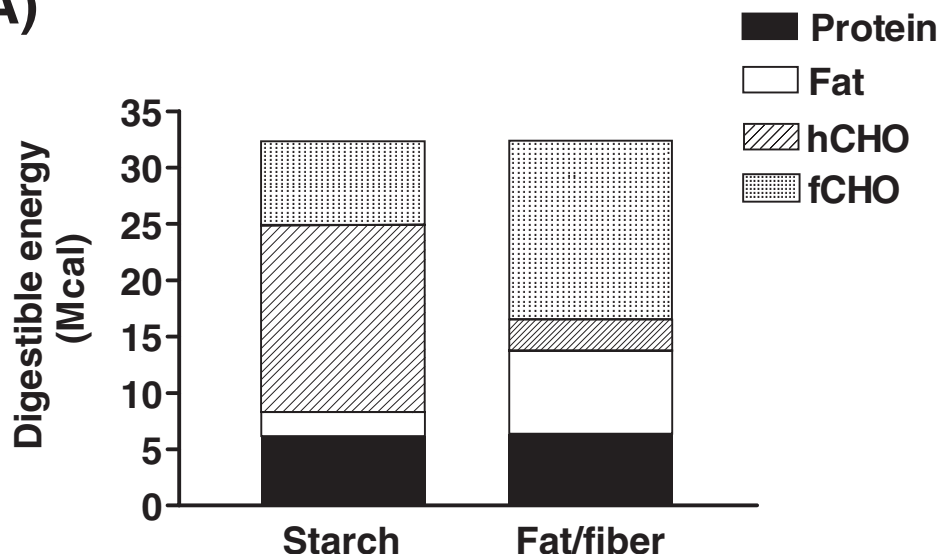
SBP or soya hulls can be included as a substitute for cereals (starch) in energy concentrates. Studies in horses have demonstrated that up to 3.0 g SBP per kg bwt per day (i.e. 1.5 kg for a 500 kg horse) may be fed to adult horses without any adverse effects on overall nutrient utilization or performance.^{61–63} Indeed, as discussed in Chapter 5.1, there may be metabolic advantages of diets that include non-starch polysaccharides such as SBP, including a muscle glycogen-sparing effect during moderate and heavy exercise.

Although the feeding of straight grains or sweet feed mixes to athletic horses remains a popular practice, there is increasing emphasis on use of energy concentrates in which some starch and sugar has been substituted by fat and/or a fermentable fiber such as SBP and soya hulls (so-called 'fat and fiber' feeds). Such diets may reduce the risk of gastrointestinal disturbances and, as discussed below, are useful in the nutritional management of horses with chronic exertional rhabdomyolysis. Figure 5.3.3 compares the sources of digestible energy in a traditional race horse diet (forage plus grain) and a diet in which a fat and fiber energy concentrate is fed.

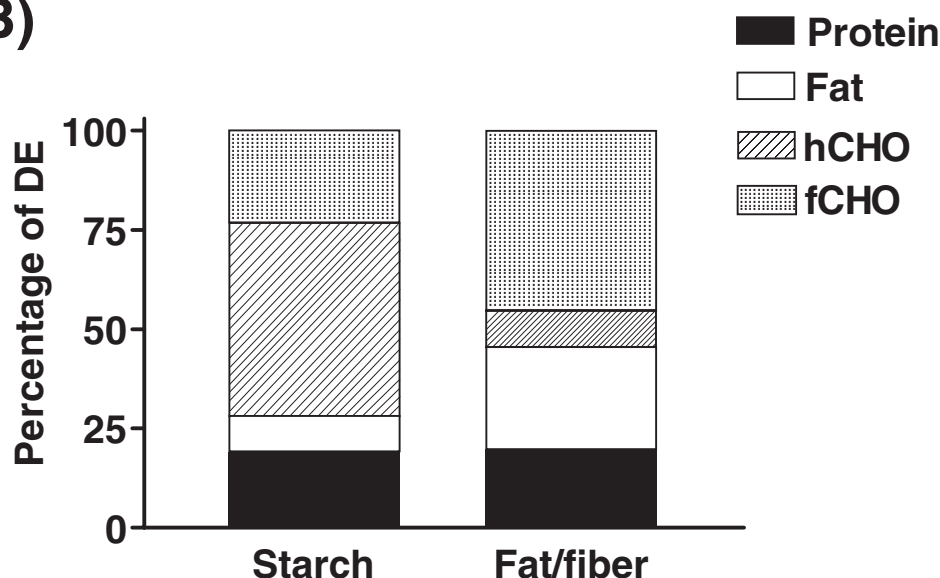
Special considerations: horses with chronic exertional rhabdomyolysis

Special considerations are needed in the selection of energy concentrates for horses with two forms of

(A)



(B)

**Fig. 5.3.3**

Graphical representation of the sources of energy in a traditional grain and hay diet (Starch) and a contemporary fat and fiber energy concentrate and hay diet (Fat/fiber) fed to a 500 kg racehorse requiring approximately 32 Mcal of digestible energy (DE) per day. Both diets include 6 kg of mid-bloom timothy hay and, on a total diet basis, provide 12% crude protein. For Starch, the horse is fed ~7 kg of an energy concentrate composed of oats (57%), cracked corn (37%), and sugar cane molasses (6%). The same quantity of energy concentrate is fed in Fat/fiber (~13% fat), the primary ingredients of which are beet pulp, rice bran, soy hulls, and vegetable oil. Graph A depicts estimates of the absolute DE provided by protein, fat, hydrolyzable carbohydrate (hCHO; starch and sugar), and fiber carbohydrates (fCHO) in the respective diets. Graph B shows the percentage contribution of the four energy sources to the total DE intake. Note the dramatic reduction in hCHO intake in the Fat/fiber diet when compared to the Starch diet.

chronic exertional rhabdomyolysis (ER); polysaccharide storage myopathy (PSSM) and recurrent exertional rhabdomyolysis (RER). Although the pathogenesis of PSSM and RER is distinctive,⁶⁴ there is increasing evidence that diets low in starch and higher in fat are beneficial in the management of both conditions.^{49,65–68}

Quarter Horses and related breeds with PSSM have enhanced insulin sensitivity, as demonstrated by a more rapid clearance of blood glucose after intravenous glucose loading⁶⁹ or the feeding of grain meal,⁷⁰ and this increase in insulin sensitivity is probably a contributing factor in the development of excessive muscle glycogen storage. Thus, diets high in starch and/or sugar may promote clinical expression of the disease by providing substrate for glycogen synthesis, and the cornerstone of dietary management for horses with PSSM is a severe restriction of hydrolyzable carbohydrate (starch and sugar) intake. There is also emerging evidence that beyond the simple removal of hydrolyzable carbohydrate from the diet, additional clinical improvement occurs when fat is added to the ration. It has been hypothesized that an increase in dietary fat alters intracellular glucose metabolism and glycogen synthesis in horses with PSSM,⁴⁹ although as yet there is no direct evidence in support of this hypothesis. Whether similar metabolic mechanisms contribute to the glycogen storage syndrome recognized in draft breeds (equine polysaccharide storage myopathy, EPSM) is not known. However, there is evidence that an increase in dietary fat is also beneficial in the management of horses with EPSM.^{65,66}

The role of diet in the pathogenesis of RER is less clear. As with PSSM, there is evidence that a reduction in starch intake and increase in fat supplementation can be beneficial.^{49,67,68,71} However, experimental studies in a small group of RER-affected Thoroughbreds have demonstrated that dietary energy source (i.e. the relative amounts of starch and sugar versus fat) is only important when total caloric intake is high (>28–30 Mcal DE per day for a 500 kg horse).^{67,68,71} Specifically, when daily calorie intake was moderate (~21–22 Mcal/day) diet composition had little influence on exercise-associated increases in plasma creatine kinase (CK) activity.^{67,68} However, post-exercise CK activity was significantly higher in horses consuming a hay and grain (starch) diet that provided 28.8 Mcal of DE per day when compared to an isocaloric diet low in starch with 20% of DE supplied by fat.⁷¹ It has been suggested that high-calorie, high-starch diets are associated with a more nervous disposition in horses. This association may explain the

relationship between dietary starch level and subclinical or clinical tying up in RER-susceptible horses given that stress and excitement are apparent trigger factors for the condition.⁶⁴ In support of this hypothesis, RER-affected horses fed the low-starch, high-fat diet had lower resting heart rates and, subjectively, a less excitable temperament when compared to observations made in the same horses while consuming the high-starch diet.^{67,68,71}

In general, there should be a reduction in hydrolyzable carbohydrate intake and increased reliance on fat and fermentable fibers as energy sources. With respect to PSSM, the diet should be devoid of grain (starch) and sugar such that on a total diet basis, less than 15% of DE is supplied by hydrolyzable carbohydrate. There is some controversy with respect to the amount of supplemental fat required for management of PSSM.⁴⁹ A suggested target is 20% of DE as fat; however, in some horses this can be difficult to achieve at very high daily DE intakes (>30 Mcal/day). 'Easy keeper' horses with PSSM that are only in light work will maintain condition on a diet that is predominantly medium-quality hay (supplied at approximately 1.5% of bwt), with a small quantity of fat (e.g. 0.5–1 kg rice bran per day or 1/2–1 cup of oil) and a vitamin–mineral supplement. Lush pasture and legume hays (alfalfa) should be avoided because these forages can supply a substantial quantity of sugar. Possible options for meeting the energy needs of affected horses in harder training include the provision of larger quantities of rice bran (e.g. 1–2 kg/day), a combination of hay pellets and vegetable oil (up to 100 g per 100 kg bwt/day), or a 'fat and fiber' energy concentrate. As mentioned, the latter utilizes a combination of vegetable fats (oils and/or rice bran) and fermentable fiber (SBP and/or soya hulls). Some of these feeds provide less than 10% of DE from hydrolyzable carbohydrate and 20% of DE as fat.

A typical race horse with RER is consuming greater than 30 Mcal of DE per day, with much of the energy supplied by starch. Indeed, it is not uncommon for race horses to consume 5–8 kg/day of a grain concentrate and this level of starch intake appears to contribute to an increased frequency of tying-up episodes.^{49,71} Therefore, as with PSSM, a lower-starch, higher-fat and fermentable fiber diet is indicated in the management of horses with RER. One recommendation for race horses in intense work is a diet that provides no more than 20% of DE from hydrolyzable carbohydrate, with a minimum of 20% DE from fat (Fig. 5.3.3).

These dietary interventions are not a panacea for the prevention of PSSM and RER. A number of other management changes are needed for the successful management of horses with chronic ER. For example, along with strict dietary control, daily exercise is critical to the successful management of horses with PSSM, while strategies for reduction of stress and excitability are important in the management of RER-susceptible horses.

Putative ergogenic feeding strategies and supplements

Beyond provision of a diet that meets the energy and nutrient requirements of an athletic horse, horse owners often apply different feeding strategies or administer a variety of nutritional supplements in an attempt to enhance a horse's performance during competition. The term *ergogenic* means 'work generating';⁷² thus, an ergogenic nutritional supplement or feeding manipulation is one that enhances work performance (e.g. an increase in speed, endurance, or strength). A plethora of nutritional supplements are marketed for use in horses, often on the basis of performance enhancement. However, with rare exceptions, there is little or no scientific basis for these claims because there are no data available regarding the efficacy of a given supplement in horses. Instead, the rationale for use is most often based on data from studies in humans or other species.⁷³

This section provides a brief overview of the effects of selected feeding strategies or nutritional supplements that have been purported to enhance athletic performance of horses. The reader is also referred to Chapter 5.1 for discussion of the effects of fat supplementation, dietary protein content, and pre-exercise feeding on exercise metabolism and performance.

Manipulation of carbohydrate supply

In humans, it is universally accepted that carbohydrate availability to skeletal muscle is an important determinant of exercise performance, particularly during moderate-intensity exercise lasting 1 hour or more.^{74,75} Low muscle glycogen concentration before exercise is associated with decreased performance, whereas high muscle glycogen content enhances endurance performance.^{74,76} Similarly, an increase in

blood glucose availability by ingestion of glucose or glucose polymers before and/or during exercise enhances the performance during prolonged moderate-intensity exercise.^{75,76} Therefore, feeding strategies that increase pre-exercise muscle glycogen content and enhance blood glucose supply to skeletal muscle during exercise are ergogenic in human athletes. This is especially true for events lasting more than 60–90 min.

The effect of carbohydrate supply on exercise performance in horses is less well studied but, similar to humans, there is evidence that glucose availability and muscle glycogen content are important determinants of performance during moderate and intense exercise. Time to exhaustion in horses running at 6–7 mph was decreased by 35% when pre-exercise muscle glycogen content was 70% lower than normal.⁷⁷ Anaerobic work capacity in horses, as assessed by the run time until fatigue during a 'supramaximal' treadmill exercise test, was decreased by approximately 28% when muscle glycogen content was 60–70% lower relative to a control treatment.⁷⁸ Thus, similar to humans, low pre-exercise muscle glycogen content is associated with decreased exercise performance in horses. On the other hand, an increase in blood glucose availability has been demonstrated to enhance performance in horses undertaking moderate-intensity exercise. In two studies of horses running on a treadmill at 50–60% of $\dot{V}O_{2\max}$, the time to fatigue was increased by 14–20% when glucose availability was increased by intravenous administration of glucose (2–3 g/min).⁷⁹ These data have generated interest in the development of nutritional strategies for horses that optimize pre-exercise muscle glycogen content or glucose availability during exercise.

Muscle glycogen storage

In humans, the term 'glycogen loading' refers to maximization of muscle glycogen stores prior to a competitive event in which performance is limited by the depletion of muscle glycogen stores. The original CHO loading protocols, pioneered by Bergström & Hultman,⁷⁴ involved a 3–4-day depletion phase of hard training and a low-carbohydrate diet, followed by a 3–4-day loading phase of high carbohydrate intake and exercise taper. This protocol resulted in a more than 50% increase in glycogen content; hence the term 'muscle glycogen supercompensation'. More recent studies have shown that well-trained athletes are able to achieve similar muscle glycogen supercompensation without the need to undertake a glycogen

depletion phase.⁸⁰ Accordingly, the more practiced method for glycogen loading in human athletes involves 3 days of exercise taper and high carbohydrate intake (7–10 g/kg bwt per day).⁸¹

Research in horses has indicated that only modest (~10%) increases in muscle glycogen content can be achieved through dietary manipulation.^{77,82,83} For example, Essén-Gustavsson et al⁸³ reported a 12% increase in the resting muscle glycogen content of Standardbred horses fed a diet that provided approximately 2 kg/day of starch and sugar when compared to an isocaloric diet that provided about 1.3 kg/day of hydrolyzable carbohydrate. An increase in muscle glycogen content of this magnitude was not associated with improved performance during moderate-intensity exercise.^{82,83} These observations notwithstanding, a number of glycogen or 'carbo' loader products are marketed for use in athletic horses.

One possible reason for the apparent disparity in capacity for glycogen supercompensation between humans and horses is a difference in hydrolyzable carbohydrate intake. In humans, a daily carbohydrate intake of 7–10 g/kg bwt is required for a substantial increase in muscle glycogen content.⁸¹ For a 500 kg horse, an equivalent carbohydrate dose would require the consumption of 7–10 kg of oats (~50% starch) per day. Such a high grain (starch) intake is not realistic for most horses, nor is it recommended given the risks of gastrointestinal dysfunction associated with high starch intake.

Although an increase in muscle glycogen content is perhaps not achievable in horses, it is still desirable to apply dietary and management practices that optimize glycogen resynthesis after exercise such that low glycogen does not prevail at the start of the next exercise session. Following exercise in horses that depletes muscle glycogen content (middle gluteal m.) by greater than 50–60%, complete glycogen replenishment is achieved by 24 hours when glucose (6 g/kg) is administered intravenously.^{78,84} In contrast, oral administration of a glucose polymer (3 g/kg bwt) within 60 min of the completion of glycogen-depleting exercise does not accelerate glycogen replenishment.^{85,86} Similarly, when horses are fed meals with high hydrolyzable carbohydrate content (grain), the rate of muscle glycogen replenishment is not different⁸⁷ or only slightly faster⁸⁸ when compared to horses fed a hay diet. Studies in humans, on the other hand, have shown that carbohydrate ingestion during the post-exercise period substantially enhances the rate of muscle glycogen resynthesis.⁷⁵ Over a 6–12-hour period following exercise, the ingestion of carbohy-

drate at a rate of 0.7–1.0 g/kg bwt every 2 hours results in muscle glycogen synthetic rates of 5–8 mmol/kg/h and complete glycogen replenishment is achieved within 24 hours. By comparison, the maximum rate of muscle glycogen synthesis in horses after exercise that results in a 40–70% decrease in glycogen content is approximately 1.5 mmol/kg/h,^{87–89} and as much as 48–72 hours is required for complete replenishment.^{88,89} As discussed, the horse appears to have a limited capacity for the digestion of hydrolyzable carbohydrates and this may limit systemic glucose availability, thereby restraining the rate of muscle glycogen resynthesis.

The performance implications of the slow rate of glycogen replenishment in horses are likely dependent on the type of athletic activity. In a study of Thoroughbreds fed a hay and grain concentrate diet, the muscle glycogen loss sustained during training gallops (19–25% decrease) was fully restored within 2–3 days.⁹⁰ Thus, in situations where intense exercise bouts occur at 3-day intervals, as is typical in the conventional training of Thoroughbred race horses, muscle glycogen content can be well maintained. However, for horses competing in multi-day events (e.g. three-day event) or multiple heats on a single day (e.g. Standardbred race horses), inadequate glycogen replenishment may adversely affect subsequent exercise performance. For these horses, small grain meals (e.g. 1–1.5 kg for a 500 kg horse) can be provided at frequent intervals (e.g. every 3–4 h) during the first 12 hours of recovery. Limiting the size of the grain meals and increasing the frequency of feeding should minimize the risks of digestive disturbance.

Enhancement of blood glucose supply during exercise

The only practical means for enhancement of blood glucose availability during exercise is the pre-exercise feeding of a meal high in starch and/or sugar, or the intragastric administration of a glucose or glucose polymer solution. For endurance horses, similar strategies could be applied at rest stops during races. However, there is some controversy regarding the merits of these pre-exercise dietary interventions. It has been argued that the suppression in lipid oxidation associated with pre-exercise carbohydrate ingestion may be detrimental to performance because an accelerated rate of carbohydrate oxidation will result in premature depletion of endogenous carbohydrate stores (see Chapter 5.1). Certainly, there is evidence

that the hyperglycemia and hyperinsulinemia consequent upon grain ingestion or intragastric glucose administration alter substrate selection during moderate intensity exercise. The consumption of a grain meal (~2 kg corn) 2 hours before exercise⁵ or the oral administration of glucose (2 g/kg bwt) 1 hour pre-exercise⁹¹ increases the rate of blood-borne glucose utilization and the rate of whole-body carbohydrate oxidation in horses during treadmill exercise at 50–55% $\dot{V}O_{2\text{ max}}$. Conversely, the rate of whole-body lipid oxidation is suppressed under these conditions,^{5,91} most probably as a result of an insulin-induced suppression in lipolysis.

It must be emphasized that the effects of pre-exercise carbohydrate ingestion (grain meals or glucose solutions) on exercise performance have not been determined in horses. Although speculative, it is possible that the pre-exercise ingestion of hydrolyzable carbohydrate is beneficial for events requiring moderate and intense exercise. However, for more prolonged, lower-intensity exercise, such as that required of the endurance horse, the suppression in lipid oxidation associated with pre-exercise carbohydrate ingestion may be detrimental to performance (see Chapter 5.1). Therefore, for endurance events it is recommended that no grain be fed in the 3-hour period before competition exercise. Further research is required to determine the metabolic and performance effects of various pre-event (and mid-event) feeding strategies.

Alterations in dietary fiber intake

The horse's large intestine contains a fluid volume equivalent to 8–10% of bodyweight, with 10–20% of total body sodium, potassium, and chloride.^{92,93} There is limited evidence that a portion of this fluid can be absorbed during prolonged exercise, thereby partially offsetting sweat fluid losses.⁹² Accordingly, some nutritionists advocate a high-fiber diet for endurance horses because such diets should increase the size of the hindgut fluid reservoir. Indeed, Meyer et al⁹² demonstrated that feeding a high-fiber diet (hay only) when compared to a low-fiber diet (a complete feed composed of grain, bran, and beet pulp) to ponies resulted in greater water content of the large intestine (183 and 101 mL/kg bwt for the high- and low-fiber diets, respectively). Warren and co-workers⁹³ also reported an approximately 15% increase in estimated gastrointestinal tract fluid volume when horses were fed a high-fiber (54% neutral detergent fiber (NDF), 31% ADF) when compared to a low-fiber diet (31%

NDF, 19% ADF). Whether such an increase in gut fluid volume is beneficial to thermoregulatory function and performance is unclear. In the study by Warren and co-workers,⁹³ neither the loss of bodyweight nor the decrease in plasma volume differed between dietary treatments during 45 min of low-intensity exercise. Furthermore, as discussed by Kronfeld,¹⁷ the putative benefits of a high-fiber diet in terms of improved water balance and thermoregulatory function must be weighed against energetic disadvantages associated with an increase in hindgut weight (bowel ballast). For example, in a 500 kg horse, an extra 4 kg in hay intake can be estimated to increase bowel ballast by between 10 and 24 kg. On balance, there is little justification for very high-fiber diets in endurance horses and, as for other equine athletes, a diet that provides roughage at about 1.5% of bodyweight is recommended.

Anecdotally, many race horse trainers limit hay intake in the day leading up to races or, alternatively, eliminate hay from the diet and instead feed a 'complete' beet pulp-based diet. There is some evidence that a short-term reduction in forage intake is beneficial in horses undertaking high-intensity exercise. When compared to ad libitum hay consumption, restricting hay intake to ~1% of bodyweight for a 3-day period before a treadmill exercise test (2 min at 115% $\dot{V}O_{2\text{ max}}$) resulted in a 2% decrease in bodyweight and a reduction in anaerobic energy expenditure during exercise, as evidenced by reduced oxygen deficit and plasma lactate concentrations. The reduction in bodyweight was attributed to a decrease in bowel ballast (gut fill).⁹⁴ The practical implications of these findings are uncertain given that many race horses do not consume more than 1% of bwt as roughage. Furthermore, a more drastic reduction in roughage intake (e.g. less than 0.75% bwt) is not recommended because, as previously discussed, low-fiber diets may predispose horses to gastrointestinal dysfunction (e.g. gastric ulcers, colic).

Nutritional ergogenic supplements

Although electrolyte, vitamin, and mineral (especially iron) supplements are the most widely used in horses, the use of supplements touted to enhance performance is also common.⁷³ Yet, very few of these substances have been the subject of scientific studies designed to evaluate their metabolic and performance effects. The following discussion is restricted to some supplements that have been evaluated in the horse.

Creatine

Creatine (methylguanidine acetic acid) is a compound derived from amino acids that is stored primarily in skeletal muscle at typical concentrations of 100–150 mmol/kg dry weight (dw) of muscle. About 60–65% of this creatine is phosphorylated. Creatine phosphate (CP) provides a rapid but brief source of phosphate for the resynthesis of adenosine triphosphate (ATP) during intense exercise and therefore helps to maintain normal ATP/ADP homeostasis. Other functions of CP metabolism include the buffering of hydrogen ions produced during anaerobic glycolysis.⁷² As both ADP and hydrogen ion accumulation are factors that may contribute to development of fatigue during sprint exercise, the size of the skeletal muscle CP store may be an important determinant of performance during high-intensity exercise. Therefore, nutritional manipulations leading to increases in total muscle creatine and CP might be expected to have an ergogenic effect during intense exercise.

The use of creatine supplements by human athletes is widespread; according to one estimate, 80% of the athletes competing in the 1996 Olympic Games were using a creatine supplement.⁹⁵ There is support for an ergogenic effect of creatine supplementation in human athletes engaged in repeated sprints, probably related to an increase in the rate of CP resynthesis during recovery between bouts of exercise. However, oral creatine supplementation is not considered ergogenic for single-bout or first-bout sprints, or for prolonged, sub-maximal exercise. The evidence is inconclusive for effects on muscle strength although creatine supplements are widely used by body builders and weight lifters.⁹⁶

Studies in humans have demonstrated that a daily creatine dose of 20–25 g (~250 mg/kg bwt/day), divided into four doses, results in increased muscle creatine concentrations, with an apparent upper limit in creatine storage of 150–160 mmol/kg dw. About 20% of the increased muscle creatine content is stored as CP and saturation occurs 2–3 days after the start of supplementation.⁹⁶ The increase in muscle creatine content is greatest in those subjects with a low initial concentration. Lower daily doses of 3 g/day (~40–44 mg/kg bwt) will achieve a slower loading over 14–28 days and elevated muscle creatine stores can be maintained by continued daily supplementation of 2–3 g creatine.⁹⁷ Creatine is transported into muscle against a high concentration gradient, via saturable transport processes that are stimulated by exercise⁹⁸ and by insulin.⁹⁹

There are two published reports of oral creatine supplementation in horses.^{100,101} Sewell & Harris¹⁰¹ demonstrated that, in contrast to humans and dogs, creatine is poorly absorbed in the horse. The intragastric administration of 50 mg Cr per kg bwt resulted in an increase in plasma creatine concentration from 40 to 100 fmol/L after 4–6 h. By comparison, the same dose in humans results in plasma concentrations of 800–1000 fmol/L.⁷³ Furthermore, the administration of creatine at 150 mg/kg per day (divided into three doses) for 13 days had no effect on muscle creatine content.¹⁰¹ In a randomized, crossover design, Schuck et al¹⁰⁰ fed Standardbred trotters 25 g creatine monohydrate twice daily (total daily dose of ~100–120 mg/kg bwt) for 14 days. Before and after the period of supplementation, horses completed an incremental treadmill exercise test until exhaustion. There was no significant effect of supplementation on plasma or muscle creatine concentrations, nor an effect on treadmill run time and the muscle metabolic response to exercise.¹⁰⁰ Thus, creatine supplementation in the horse at dosages shown to be effective in humans has failed to result in an increase in muscle creatine content. Without a change in muscle creatine content, creatine supplementation is unlikely to exert an ergogenic effect in horses. The reason for the apparent low bioavailability of orally administered creatine in horses has not been determined. It is possible that, as an herbivorous animal, the horse's gastrointestinal tract is not adapted to the absorption of creatine.

L-Carnitine

L-Carnitine is a component of the enzymes carnitine-palmitoyltransferase I, carnitine-palmitoyltransferase II, and carnitine-acylcarnitine translocase that are involved in the transport of long-chain fatty acids across the inner mitochondrial membrane.¹⁰² As such, long-chain fatty acid oxidation is carnitine-dependent and, therefore, it has been proposed that increased availability of L-carnitine by supplementary ingestion might upregulate the capacity to transport fatty acids into the mitochondria and increase fatty acid oxidation.⁷² This augmentation in fat oxidation could be of benefit during endurance exercise. Another role of carnitine is to act as a 'sink' for acetyl-CoA units produced during high-intensity exercise. The conversion of acetyl-CoA to acetylcarnitine maintains CoA availability and decreases the ratio of acetyl-CoA:CoA. As such, an increase in carnitine availability could enhance substrate flux through the citric acid cycle and increase the activity of pyruvate dehydrogenase,

which is otherwise inhibited by high levels of acetyl-CoA. These mechanisms would serve to increase oxidative metabolism of glucose, decrease lactate production, and perhaps enhance performance during exercise tasks that might be limited by excess hydrogen ion and lactate accumulation.⁷²

However, the weight of evidence from human studies indicates that oral carnitine supplementation has no effect on muscle carnitine concentration. In addition, there is no evidence that muscle carnitine content limits fat oxidation other than in patients with inborn errors in metabolism that result in inadequate muscle carnitine. This is also likely to be the case in horses as the carnitine content of equine skeletal muscle is 2–3-fold higher when compared to human muscle.¹⁰³ Supplementation studies in humans have failed to demonstrate an effect of carnitine on measures of fat oxidation or muscle metabolism during exercise.^{72,102}

Studies in humans¹⁰⁴ and in horses^{105,106} have demonstrated that the oral bioavailability of L-carnitine is poor. In horses, large oral doses of L-carnitine (10–60 g) are required to effect an approximate doubling in plasma carnitine concentration.¹⁰⁵ Importantly, supplementation at these levels for 58 days had no effect on muscle carnitine content. In a subsequent study, intravenous doses of 10 g L-carnitine were administered daily for 26 days.¹⁰⁶ These infusions resulted in peak plasma carnitine concentrations 30-fold higher when compared to pre-injection values and concentrations remained threefold higher after 6 h. Yet, there was no change in muscle carnitine content. Thus, from the available data there is no evidence that muscle carnitine content in horses is enhanced as a result of oral or intravenous L-carnitine supplementation.

Although the weight of evidence suggests that L-carnitine is unlikely to be ergogenic in horses, a recent study has provided evidence that supplementation during conditioning may augment training-associated skeletal muscle adaptations.¹⁰⁷ In a small group of 2-year-old Standardbred horses subjected to a 10-week conditioning program, supplementation with L-carnitine (10 g/day per os) was associated with significant increases in the percentage of type IIA muscle fibers and the intensity of periodic acid–Schiff staining (an indicator of intrafiber glycogen content) when compared to untreated control horses.¹⁰⁷ The mechanism of these effects is unclear and somewhat perplexing, given that previous studies have failed to demonstrate a change in muscle carnitine content in horses receiving an identical carnitine dosage. Further

studies are required to determine the performance implications of these apparent carnitine-induced muscular adaptations.

Amino acids

Amino acid or ‘refined’ protein supplements are often touted for their ability to ‘build’ muscle mass or, in the case of the branched-chain amino acids (BCAA) (leucine, isoleucine, valine), enhance endurance performance by modifying factors that contribute to central fatigue.^{108,109} In addition, it has been proposed that BCAA supplementation during exercise may provide carbon intermediates for the citric acid cycle at a time when endogenous carbohydrate reserves are depleted, thereby delaying the onset of fatigue.⁷²

The ‘central fatigue’ hypothesis proposes that increased brain serotonin contributes to fatigue development during prolonged moderate-intensity exercise.¹⁰⁹ The increase in brain serotonin synthesis occurs as a result of increased transport of free (unbound) tryptophan across the blood–brain barrier. Key to this increase in tryptophan uptake is an increase in the plasma ratio of free tryptophan to BCAA,¹⁰⁹ which may increase for two reasons. First, as blood free fatty acid (FFA) concentration rises during exercise, the FFA compete with tryptophan for binding sites on albumin and the FFA displace some of the tryptophan molecules from albumin; therefore, free tryptophan concentration increases. Second, an increase in the oxidation of BCAA in muscle results in a decrease in blood BCAA concentration. As BCAA and tryptophan compete for carrier-mediated entry into the central nervous system, an increase in the free tryptophan-to-BCAA ratio leads to increased tryptophan transport. Therefore, it has been theorized that BCAA supplementation could reduce the exercise-induced increase in brain tryptophan uptake and thus delay fatigue.¹⁰⁹ Interestingly, when horses were infused with tryptophan (100 mg/kg, i.v.) during submaximal exercise, run time to fatigue was decreased by ~15% relative to the placebo treatment, providing evidence that an increase in circulating tryptophan adversely affects endurance performance in horses.⁷⁹ However, the oral ingestion of a tryptophan solution that markedly increased plasma free tryptophan concentration and estimated brain tryptophan uptake had no effect on time to exhaustion in exercising humans.¹¹⁰ Furthermore, in this and other studies, the ingestion of large quantities of BCAA also had no effect on endurance performance.^{72,110} These data cast

some doubt on the 'central fatigue' hypothesis and suggest that BCAA supplementation is not ergogenic during dynamic exercise in humans.

BCAA supplements are marketed for use in horses, but there are no published data regarding their effects on exercise performance per se. Glade¹¹¹ reported that BCAA supplementation mitigated the increase in blood lactate during exercise. However, the exercise test involved treadmill walking and the applicability of these results to equine athletic activities is questionable. More recent studies have failed to demonstrate a beneficial effect of BCAA supplementation in horses. The administration of a mixture of L-leucine (9 g), isoleucine (4.5 g), and L-valine (9 g) to Standardbreds 1 h before training had no measurable effect on energy metabolism during intense exercise.¹¹² Similarly, there were no changes in plasma biochemical variables during and after exercise in horses fed BCAA three times per week for 5 weeks.¹¹³

Several studies in humans have demonstrated that increased amino acid availability early in the post-exercise period modifies protein metabolism in skeletal muscle.^{114–117} Specifically, hyperaminoacidemia resulting from ingestion or intravenous infusion of amino acids increases post-exercise muscle protein synthetic rate and prevents the exercise-induced increase in protein degradation. Thus, post-exercise amino acid or protein supplementation may promote

anabolism in skeletal muscle during conditioning. Neither the effects of exercise in muscle protein metabolism nor the effect of post-exercise amino acid or protein supplementation on these processes have been investigated in horses.

Antioxidants

As discussed, exercise has been linked with an increase in free oxygen radical species that may cause cellular damage. Thus, it has been postulated that supplementation of athletes with antioxidants such as vitamin E or vitamin C will increase antioxidant status and confer protection against free radical-associated damage. Although there is evidence in humans that antioxidant supplementation provides some degree of protection during periods of increased stress, such as a sudden increase in training load (see Packer 1997 for review),³² overall the literature on the effects of antioxidant supplementation is confusing and no clear recommendations have emerged for supplementation strategies in human athletes. Similarly, the performance effects of large-dose antioxidant supplementation in horses are unclear. On the other hand, there is evidence that antioxidant supplementation of horses with recurrent airway obstruction improves antioxidant status and moderates airway inflammation.¹¹⁸

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Body fluids and electrolytes: responses to exercise and training

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The exercising horse produces a tremendous amount of metabolic heat. This byproduct of the transduction of potential energy into kinetic energy can raise core body temperature in the horse from 37°C at rest to temperatures exceeding 42°C in a matter of minutes.^{1–6} Normal cellular function, however, requires active and efficient ways to keep core body temperature within narrow limits. To do this a horse must move heat produced in the muscles to the periphery.^{1,2,4–6} Other mammalian species, like the rabbit and dog, have an enhanced countercurrent brain bloodflow mechanism that couples with panting to provide for cooling of the brain while permissively allowing heat storage in the rest of the body and a rise in body temperature.^{5,7,8} Horses and humans are the only species that cool primarily through the evaporation of sweat.^{1,5,7,8} Sweating can produce tremendous fluid and electrolyte losses that, if uncompensated for, can lead to cardiovascular and thermoregulatory instability. These fluid deficits and their effects are even more pronounced during endurance exercise and exercise performed during periods of high environmental temperature and humidity.^{1–3,5–7} Recent work focused on preventing thermal injuries in horses has documented findings similar to those published in the human sports medicine literature: namely, that maintenance of fluid and electrolyte balance prevents dehydration and provides thermoregulatory and cardiovascular stability.^{3–12} Data demonstrate that optimal fluid and electrolyte balance delays the onset of fatigue. The present chapter reviews the current literature on the effects of exercise on fluid balance and renal function in horses.

Body fluid compartments

Like all animals, the body of the horse is primarily composed of water and electrolytes. Those solutions are compartmentalized within and outside the cells. This combination of intracellular and extracellular water is referred to as the total body water (TBW). The TBW accounts for 50–70% of bodyweight, or 250–350 kg of the bodyweight of a typical 500 kg horse.^{1,2,9} TBW can be measured using various indicator dilution techniques, stable isotope techniques, and bioelectric impedance technologies.^{1,2,9} Each of these techniques has advantages and disadvantages, with the use of stable isotope infusion being one of the most accurate and bioelectric impedance technology the least reliable. The TBW is divided by cell membranes into two primary fluid compartments, the intracellular fluid compartment (ICF) and the extracellular fluid compartment (ECF).^{1,2,8} Approximately two-thirds of the TBW (~200 L) is contained within the cells of the body, leaving one-third of the water in the ECF space (~100 L). According to Carlson,² the latter is further compartmentalized into fluid contained within the vascular space, the interstitial fluid space, the lymphatics, and transcellular fluids. This last category includes the fluid content of the gastrointestinal tract, which represents a large reservoir of fluid.²

The vascular space or total blood volume is filled with a mixture of fluid and cells, the latter primarily red blood cells, but also including white blood cells and platelets. Thus, the total blood volume (BV) is the combination of the plasma volume (PV) and the red cell

volume (RCV), or, stated as a formula:² $BV = PV + RCV$. Blood volume varies from breed to breed, with age, body composition, hydration status, and training status.^{2,13} Across breeds, studies have reported total blood volumes ranging from 61 mL/kg in draft horses to 137 mL/kg in race horses.^{1,2,9} In the average 450 kg horse, total BV would be about 36 L, PV ~16 L and RCV around 20 L.^{1,2,13}

The eloquent work of Persson¹³ and many others^{2,14–16} has shown that there is a strong relationship between red blood cell volume and aerobic performance in the horse. Oxygen uptake and delivery are dependent on both optimal volume to insure cardiac filling pressure and an optimal number of red blood cells to carry oxygen. Much focus has been placed on the need to have red blood cells to carry oxygen to the working muscles. However, while RCV and PV are usually looked at independently, they are interdependent in the optimization of blood flow during exercise. Blood flow can be affected by changes in viscosity; thus, too many red blood cells and not enough plasma can cause a substantial change in viscosity, as well as other factors related to resistance to flow. The values for BV, PV, and RCV in the literature also vary with the differing methodologies used in different laboratories to measure and/or calculate total blood volume.^{2,13–16} For the most part, studies of equine blood volume have not used direct measurement of BV. Instead, they have generally used dye or indicator dilution techniques to measure PV and then calculated BV using the measured PV and hematocrit (HCT):^{2,13–16}

$$BV = \left(\frac{PV}{100 - HCT} \right) \times 100$$

The measurement of PV using dye or indicator dilution techniques requires the use of an indicator that stays within the vascular compartment for a long enough time to reach full steady-state distribution without substantial removal through the metabolism of the dye by the tissues.^{2,15} Ideally, this requires an indicator that binds to a large molecule that does not readily leave the vascular compartment. Two substances commonly used to measure PV in the horse are indocyanine green (IC-green or cardiac green) dye and Evans Blue dye.² While IC-green dye has been used to measure plasma volume in the horse, one must caution that, because it is rapidly cleared from the vascular compartment, it is better suited for the repeated injections required for the measurement of cardiac output. The rather short half-life of IC dye means it can be cleared before reaching full distribu-

tion, affecting the accuracy and repeatability of PV measurements in the horse. Evans Blue dye binds to albumin and thus has a relatively long half-life and stays for the most part in the vascular compartment.^{2,13,15} However, one must caution that albumin can shift out of the vascular compartment if there is an increase in hydrostatic pressure induced by manipulations such as exercise or epinephrine (adrenaline) infusion. Thus, the measurement of PV using the Evans Blue dye dilution technique requires that a horse be standing relatively quietly and unperturbed by exercise or pharmacological manipulations for the 15- to 20-min mixing period between collection of a blank plasma sample and injection of the dye, and the collection of a post-injection blood sample.^{2,15} Anything that disturbs the steady-state of the cardiovascular system affects distribution of the dye; therefore, one should view with extreme caution published studies reporting PV, BV, and RCV values calculated using a post-injection sample obtained after exercise as the readings can be skewed in two ways:¹³ first, decreases in PV due to water shifts out of the vascular compartment caused by increases in hydrostatic pressure that would give artificially high concentration of the dye; and, second, errors caused by the loss of dye from the vascular compartment due to extrusion of albumin out of the bloodstream by the same increases in hydrostatic pressure.^{3,17–20} Plasma volume can decrease 15–20% after only three 1-min steps of an incremental exercise test;¹⁷ therefore, errors in the measurement of plasma volume due to non-steady-state sampling would also affect the calculation of BV and RCV using the above formula.

Another factor that must be considered in the calculation of total BV in the horse is the splenic reserve volume. The horse is somewhat unique compared to most other mammalian species in that the spleen is a very capacious and capricious organ, storing between 6 and 12 L of red cell-rich blood at rest.^{2,13,19,20} Splenic blood typically has a hematocrit of ~65–75%.^{2,13} Thus, studies of total BV become somewhat problematic because measurement of the total circulating BV requires an accounting for the splenic reserve volume, a measurement that requires mobilization of the splenic red cell reserve. Most studies to date have utilized exercise or infusion of epinephrine (adrenaline) or an α -adrenergic agonist drug to cause splenic contraction, with a blood sample obtained for the measurement of hematocrit after the accommodation and the mixing of the extra volume of blood. Complete mixing takes only 1–2 min; however, in many studies, the hematocrit used to calculate BV and RCV was

taken at the end of or after an incremental exercise test.^{13,16} While this is an accepted way to cause splenic contraction and a viable way to *estimate* the contribution of the splenic reserve to the total circulating blood volume, the resulting hematocrit values are skewed upward by the dynamic fluid shifts caused by the changes in flow and hydrostatic pressure induced by the exercise or pharmaceutical manipulation. Therefore, the hematocrit used to calculate total BV would reflect both the contribution of splenic reserve mobilization and reductions in plasma volume and would be an overestimation of total blood volume. This is essentially an offset error and, because acute reductions in PV caused by exercise-induced fluid shifts are linked to exercise intensity,^{3,17–19} the fluid shifts that lead to this overestimation only become a problem if a study's experimental design uses different exercise intensities to measure the hematocrits used in comparisons between treatment groups or comparisons made before and after training. For example, if one calculates BV using a hematocrit obtained at the 10 m/s step of a treadmill test, it will yield a different result than the value calculated using the hematocrit collected at the 11 or 12 m/s step of an incremental treadmill test.

As mentioned above, the absolute value for resting plasma volume can be determined using Evans Blue dye. However, measurement of PV during exercise and any resulting decreases are problematic because of mixing time, the requirement for steady-state conditions, and the potential for overwhelming the vascular space with dye through repeated injections. Percent changes in PV can be measured using changes in protein concentration.¹⁷ However, because some protein leaves the vascular compartment, this method tends to underestimate the reduction in PV.¹⁷ To get around these methodological problems, studies of human athletes^{21–23} have utilized changes in hematocrit to calculate percent changes in PV (Example 1). Absolute volume changes in liters are then calculated using the previously measured absolute resting PV determined using Evans Blue dye.^{21–23} The calculation of percentage change in PV using hematocrit is feasible because red blood cells do not leave the vascular compartment like protein molecules and any change in their concentration must be due to changes in plasma volume.^{21–23} In humans, the calculation is simple and involves the use of a pre-exercise hematocrit (HCT_b) and hematocrits measured during or after exercise (HCT_a).^{21–23} For example, if the hematocrit measured before exercise (HCT_b) was 43 and the hematocrit measured in a blood sample obtained after

Example 1 Calculation of the percent change in plasma volume using uncorrected hematocrit as in humans

$$\% \Delta PV = \left(\frac{100}{100 - HCT_b} \right) \times \left[100 \times \frac{(HCT_b - HCT_a)}{HCT_a} \right]$$

$$\% \Delta PV = \left(\frac{100}{100 - 43} \right) \times \left[100 \times \frac{(43 - 45)}{45} \right]$$

$$\% \Delta PV = \left(\frac{100}{57} \right) \times \left[100 \times \frac{(-2)}{45} \right]$$

$$\% \Delta PV = (1.8) \times [-4.4]$$

$$\% \Delta PV = -7.9$$

10 min of exercise was 45, then the change in plasma volume is -7.9% . Thus, one can see that a relatively small change in hematocrit represents a much larger change in plasma volume.

However, it is important to note that the use of this formula requires that there is no addition of red blood cells to the central circulation or change in the size of the cells.^{21–23} The latter has been shown to not be a problem if exercise duration is less than 120 min.²³ The former makes the use of this formula problematic for those doing horse research because the spleen adds RBCs to the central circulation. Fortunately, McKeever et al have developed a correction factor obtained after comparing sequential blood samples taken from splenectomized and intact horses.¹⁷ These studies demonstrated that the spleen contracts very rapidly with both the extruded volume and cells accommodated and mixed with the central circulation within the first 1–1.5 min of exercise.¹⁷ Changes in hematocrit from the point of full mixing onward paralleled each other in both groups of horses. Thus, the changes in hematocrit from that point on were due to decreases in plasma volume caused by fluid shifts and loss of water from the vascular compartment.¹⁷ More importantly, the difference between the pre-exercise and the 2-min values for hematocrit in the intact horses represented an offset due to splenic reserve mobilization that could be used as a correction factor.¹⁷

Example 2 demonstrates how to use hematocrit in the horse to calculate percent changes in PV. For example, if a horse had a resting hematocrit (HCT_b) of 35 and the hematocrit measured at 2 min of exercise (HCT_{2 min}) were 55, then the difference between the two would be the calculated correction factor (HCT_{2 min}) to be used to correct all the hematocrits

Table 6.1.1 Electrolyte composition (mEq/L) of plasma, interstitial fluid, intracellular fluid (muscle), and sweat

Electrolyte	Plasma	Interstitial fluid	Skeletal muscle cell	Sweat
Cations				
Na ⁺	140	143	10	120 (range 115–150)
K ⁺	4	4.1	142	35 (range 25–50)
Ca ²⁺ (ionized)	2.5	2.4	4	10 (range 3–20)
Mg ²⁺ (ionized)	1.1	1.1	34	5
Anions				
Cl ⁻	100	113	4	150 (range 140–190)
HCO ₃ ⁻	25	28	12	
H ₂ PO ₄ ⁻ , HPO ₄ ²⁻	2	2	40	
Protein	14	0	50	
Other	7	7	84	

Example 2 Calculation of the percentage change in plasma volume using corrected hematocrit in horses¹⁷

HCT_b = 35

HCT_{raw} = 58

HCT_{2 min} = 20

HCT_a = HCT_{raw} - HCT_{2 min} = 58 - 20 = 38

$$\% \Delta PV = \left(\frac{100}{100 - \text{HCT}_b} \right) \times \left[100 \times \frac{(\text{HCT}_b - \text{HCT}_a)}{\text{HCT}_a} \right]$$

$$\% \Delta PV = \left(\frac{100}{100 - 35} \right) \times \left[100 \times \frac{(35 - 38)}{37} \right]$$

$$\% \Delta PV = \left(\frac{100}{65} \right) \times \left[100 \times \frac{(-3)}{37} \right]$$

$$\% \Delta PV = (154) \times [-5.41]$$

$$\% \Delta PV = -12.2$$

measured after the 2-min point of exercise onward. Thus, in Example 2, if the uncorrected hematocrit obtained at 15 min of exercise were 58 (HCT_{raw}), then the value for HCT_a to be used in the formula would be obtained by subtracting the correction factor from the uncorrected hematocrit.

Plasma osmolality and the concentration of key electrolytes

Normal cellular function is vitally linked to maintenance of fluid, electrolyte, and acid–base balance within a narrow range.^{2,3,23–26} Thus, the composition

of both the plasma within the vascular compartment and the fluid within the intracellular fluid space is tightly controlled.^{2,3,23–26} Key to maintenance of the internal environment is regulation of overall plasma osmotic concentration or osmolality as well as the concentration of key electrolytes such as sodium, potassium, and chloride.^{2,3,23–26} Sodium is the major anion contributing to osmolality and is the major cation in the extracellular fluid space.^{2,3,23–26} Potassium, on the other hand, is the primary cation found within the cells.^{2,3,23–26} Other important cations include calcium and magnesium, both primarily intracellular ions.^{2,3,23–26} When considering exercise, it is the calcium found within the muscle in the tubular sarcoplasmic reticulum that is important.^{2,3,23–26} This calcium plays a vital role in the process of excitation contraction coupling. Magnesium found within the cells is an important cofactor in many of the reactions involved in various metabolic pathways.^{2,3,23–26} Major anions include chloride, bicarbonate, and the phosphates.^{2,3,23–26} Normal values for resting concentrations of the major electrolytes found in plasma, interstitial fluid, intracellular fluid, and sweat can be found in Table 6.1.1. All of the electrolytes contribute to the osmotic concentration of the body fluids, a variable that is tightly regulated to prevent cell dehydration or cell swelling.

The osmotic concentration or osmolality of the plasma is essentially the same as that of the rest of the interstitial fluids.^{27,28} Normal plasma osmolality in the horse and most other mammals averages 290 mOsm/L.² Plasma osmolality is the total number of dissolved particles in solution, independent of the elemental species making up that solution.²⁷ Plasma osmolality reflects the osmolality of both the extracellular fluid space and the intracellular fluid space²⁷ and

is important for two reasons. First, large molecules in solution exert osmotic force across semipermeable membranes such as capillaries and cell membranes. Thus, plasma osmolality is a measure of the total 'osmotic pull' or osmotic force that is exerted by the sum of freely moving particles in solution exerting an effect on water in surrounding tissues.^{27,28} Because water tends to move down a concentration gradient from an area of low concentration to an area of high concentration, an increase or decrease in plasma osmolality has the capacity to alter normal cellular function dramatically by causing fluid shifts into and out of the cells, shifts that can decrease cell function.^{27,28} Second, a change in osmolality reflects expansion or contraction of the extracellular fluid compartment.^{27,28} Optimal cardiovascular function is highly dependent on fluid volume status and mechanisms associated with the maintenance of plasma osmolality and extracellular fluid volume serve as one of the first lines of defense in the regulation of cardiac filling volume and pressure and ultimately mean arterial pressure (MAP) and the ability to perfuse the tissues.^{19,27,28}

During exercise the horse can lose tremendous volumes of hypertonic sweat presenting a serious challenge for maintaining the volume and the composition of the body fluids.^{1,2,19} Dramatic changes can compromise cellular function.²⁸ It can also compromise cardiovascular stability through a reduction in venous return and cardiac output. Therefore, it is vitally important that the body regulates plasma osmolality within very narrow limits.²⁸ Defense of osmolality is vitally intertwined with the defense of extracellular fluid volume, plasma volume, and cardiac filling pressure.^{19,28} Thus, defending plasma osmolality involves an integrative response of multiple systems including the cardiovascular, neural, endocrine, and renal systems.^{6,10,19,27-30} Changes in plasma osmolality are sensed by specialized cells within the supraoptic and paraventricular nuclei of the hypothalamus.^{19,27-29} These osmoreceptors are very sensitive and changes in plasma osmolality as small as 2 mOsm/L can evoke a change in the synthesis and secretion of the hormone arginine vasopressin (anti-diuretic hormone) by the posterior pituitary.²⁹ Changes in circulating vasopressin concentration occur rapidly and can cause dramatic alterations in renal handling of water within minutes, thus correcting volume deficits and swings in the concentration of osmotically active substances through losses of plasma water or electrolytes in the sweat.^{29,30} Vasopressin also stimulates thirst and drinking, which ultimately affects water balance and osmolality.²⁹

Plasma concentration versus plasma content

When considering the effects of acute exercise on changes in key electrolytes one must distinguish between changes in the concentration versus changes in the total content of those electrolytes.^{23,31} By definition, the concentration of a substance is the amount of solute in a given volume of solvent. Content, on the other hand, is the total amount of that solute in the fluid compartment or body depending on the focus of analysis. For example, normal plasma concentration of sodium is 140 mEq/L or, put another way, there are 140 mEq of sodium per liter of plasma. Plasma content of sodium would be obtained by multiplying the concentration of sodium by the plasma volume:^{23,31}

$$\frac{140 \text{ mEq}}{\text{Liter}} \times \frac{26 \text{ Liters}}{1} = 3640 \text{ mEq}$$

Calculation of changes in the content of key electrolytes and other substances allows one to determine if changes in the concentration of a substance is the result of addition or loss of the substance or just due to changes in plasma water.^{23,31} When viewed on a whole body level, changes in content allow one to make calculations giving insight into how concentrations of tightly regulated plasma constituents are affected through routes of intake or loss that affect whole body balance of said constituent.^{23,31} Acutely, calculation of relative or percent changes in the content of plasma volume and the plasma constituents gives one insight into the dynamic changes that occur in response to the challenge of exertion.^{23,31} To that end, human exercise physiologists for years have used key formulae to calculate percentage changes in plasma volume and percentage changes in the content of various plasma constituents during exercise (Example 3).²¹⁻²³ If one knows the resting plasma volume, then once one calculates the percentage change in the content of a plasma constituent, one can calculate the total amount of that substance lost during exercise from the vascular compartment.^{23,31}

On a practical level, calculation of changes in the content of various plasma constituents can give insight into their disposition. An example of this would be an examination of sodium and chloride losses during short-term versus endurance exercise. Plasma sodium and chloride concentrations are held within very narrow limits. During short-term exercise plasma sodium and chloride concentrations undergo minimal changes.³¹ However, the plasma content of sodium

Example 3 Calculation of percent change in the content of a plasma constituent using corrected hematocrit³¹

HCTb = Resting hematocrit

HCTa = Corrected hematocrit

Cnb = Resting concentration

Cna = Post-concentration

Co = Content of solute

$$\% \Delta Co = \frac{Cna - [HCTa(100 - HCTb) \times (Cnb)]}{HCTa(100 - HCTb) \times (Cnb) / HCTb(100 - HCTa)} \times 100$$

and presumably chloride decreases suggesting the fluid shifts that occur during short-term exercise involve an isotonic shift of fluid.³¹ During longer-term exertion there can be minimal changes in plasma sodium concentration but content can change dramatically.^{23,31} With chloride there is a dramatic disproportional decrease both in the plasma concentration and content due to large amounts lost in the sweat.^{23,31} Measuring total content lost gives a more complete picture of how much supplementation must occur to replenish exercise-related losses. When one looks at changes in plasma potassium concentration and content one sees a different picture. Both the concentration and content of potassium go up during high-intensity exercise. When one looks at the change in content one can see that the change in concentration is due to both the loss of plasma water and the addition of potassium to the plasma when it leaks out of the contracting muscle cells.

Effects of acute exercise on fluid and electrolyte balance

Hypothetically, fluid and electrolytes can shift from the intracellular space to the extracellular space as well as between each of the compartments through active, passive, and facilitative mechanisms.^{6-8,11,18,19,23,32,33} This dynamic exchange of fluid and electrolytes between compartments moves nutrients and waste products, provides fluid and electrolytes for the production of sweat, and allows the horse to defend the internal environment of the cells.^{6-8,11,18,19,23,32,33} To

maintain cellular homeostasis the horse must regulate blood volume, blood pressure, and the osmotic composition of the intracellular and extracellular fluid compartments. Acute fluid and electrolyte shifts have differing functional significance related to the timing of the response during exercise. Early shifts appear more related to a system-wide redistribution of blood and fluid from capacitance vessels and the interstitial space so as to increase venous return and augment cardiac output.⁶⁻⁸ Later responses provide fluid and electrolytes for the production of sweat and thermoregulation.^{6,11,17,23} Finally, decreases and depletion of fluid stores lead to dehydration, thermoregulatory and cardiovascular instability, and fatigue.^{6,11,17,23} This latter challenge stimulates an expansion of plasma volume and the contents of the various electrolytes, a beneficial adaptive response known as a training-induced hypervolemia.^{6,11,17,23}

Intercompartmental fluid shifts at the onset of exercise

Senay³⁴ demonstrated, in humans, that in the first seconds at the onset of exercise, there is a rapid net movement of protein and fluid from the interstitial space and lymphatics into the vascular compartment.³⁴ This inward flux of protein and water causes a transient, short-lived increase in plasma volume, an intercompartmental shift of body fluids that couples with a redistribution of blood from the venous capacitance side of the vascular system to the arterial side to provide adequate venous return to maintain cardiac filling pressure.^{6-8,34} This important redistribution of blood and fluid from the capacitance side of the vascular system is important because of the need for extra venous return at a time when there is rapid vasodilation in the working muscles.^{6-8,34-36} Similar phenomena have been demonstrated in dogs and probably also occur in horses.^{35,36} Studies³⁷ have demonstrated a shift in the albumin to globulin ratio in the horse consistent with an inward flux of fluid from the interstitial space. At rest albumin is the primary protein found in the vascular space and globulin represents the most prevalent protein found in the lymphatics.³⁷ This dramatic change in albumin to globulin ratio suggests that the horse experiences a similar influx in fluid at the onset of exercise as described in humans.^{34,37} Plasma is also added to the central circulation upon mobilization of the splenic reserve.^{13,17} Splenic blood is rich in red blood cells, with a hematocrit between 65

and 75%.^{13,17} However, this also means that there is an addition of plasma to the central circulation. Splenic blood volume averages range from 8 to 12 L; thus, there is an addition of 1.6 to 3.6 L plasma to the central circulation in addition to the volume added by intercompartmental fluid shifts.^{13,17} This extra volume of plasma serves a role in cardiovascular control and it also increases circulating protein that provides extra buffering capacity to the central circulation.^{13,17}

Plasma volume decreases rapidly after this initial intercompartmental redistribution of water and electrolytes.^{6,17,18,23,32,33,38} These secondary fluid shifts are caused by significant increases in MAP and consequently capillary hydrostatic pressure that cause water, electrolytes, and a small amount of protein to be extruded from the vascular compartment.^{6,23} Studies of horses^{17,19,20} performing moderate incremental exercise have demonstrated that this decrease in plasma volume is rapid and intensity-dependent with a 15–20% decrease observed after only four 1-min steps of an incremental exercise test (Fig. 6.1.1). This movement of water and salts bathes the intersti-

tial space, where they can be taken up into the working muscles, used to form sweat, or returned to the vascular compartment.^{17,19,20} In mammals there is a dynamic flux of fluid into and out of the vascular compartment that is governed by Starling forces (Fig. 6.1.2).^{2,6–8,11} Net filtration and reabsorption across a vascular bed is the sum of forces affecting the movement of fluid and osmotically active substances in both the arterial and venous capillaries.^{2,6–8,11} These forces include the capillary and interstitial hydrostatic pressures and the capillary and interstitial oncotic pressures. On the arterial side of the resting capillary bed, hydrostatic pressure and interstitial oncotic pressure outweigh interstitial pressure and intravascular oncotic pressure.^{2,6–8,11} This favors a net movement of fluid out of the vascular compartment. However, resting venous oncotic forces outweigh the other forces and favor a movement of fluid back into the vascular space.^{2,6–8,11} Some fluid is not returned via the influx into the venous capillaries and is returned through the lymphatic system.^{2,6–8,11} During exercise, the balance of Starling forces is greatly affected by a larger increase in arterial hydrostatic pressure.^{2,6–8,11}

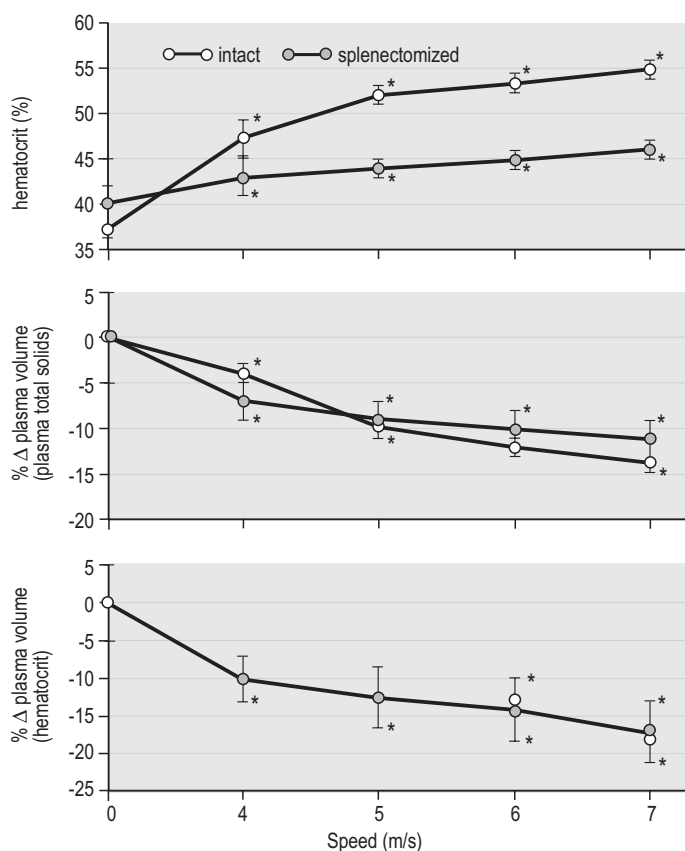
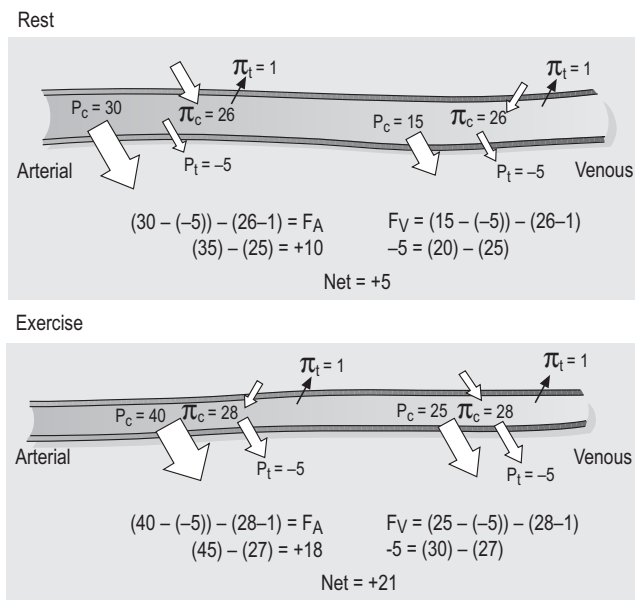


Fig. 6.1.1

Percentage change in plasma volume during incremental exercise. Values are means $\pm$ SE for hematocrit, percentage change in plasma volume calculated using total solids (i.e. protein), and percentage changes in plasma volume calculated using corrected hematocrit. (Reproduced with permission from McKeever et al.¹⁷)

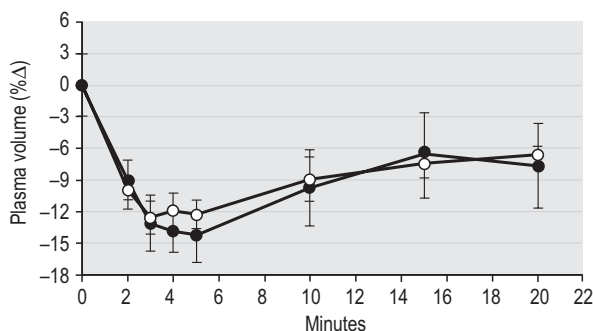
**Fig. 6.1.2**

Starling forces affecting the movement of fluid in and out of the vascular compartment. Top: At rest, the balance of forces on the arterial side of the capillary bed is positive (+10), favoring an outward movement of fluid. On the venous side of the capillary bed the balance of forces is negative (-5), favoring the inward movement of fluid. However, the net difference between the arterial and venous sides of the capillary beds is positive (+5) and thus not all the fluid is returned to the bloodstream. The excess must be returned via the lymphatic system. Bottom: During exercise, hydrostatic pressure increases in both sides of the capillary bed, enhancing the movement of fluid outward into the interstitial space for transport and for sweat production. Thus, during exercise, lymphatics play an important role in returning fluid not lost as sweat.

At the arteriole level this amounts to a ~20 mmHg increase in hydrostatic pressure enhancing the net force of fluid outward.^{2,6-8,11} On the venous side of the capillary beds, hydrostatic pressure is also elevated with a tendency for a net positive outward force. This means that more fluid is shifted into the interstitial space when compared to rest. This extra dynamic outward flux of fluid has a functional significance and is beneficial as it can either be excreted as sweat or returned to the vascular compartment via the lymphatics. The net result is a decrease in plasma volume and a dynamic flux of fluid that provides for removal of metabolic waste products and for removal of heat produced during exercise.^{2,17,20} The key here is that the decrease in plasma volume seen at the onset of exercise^{2,17,20} is dynamic and intensity-dependent (Fig. 6.1.3) and occurs before the onset of sweat losses.^{17,20} However, plasma volume decreases seen after these initial fluid shifts are the result of reductions in total body water caused by sweating.^{1,2}

Fluid and electrolyte losses associated with longer acute exercise

Exercise continued beyond a few seconds causes pronounced hemodynamic changes and, because of sweat production, fluid and electrolyte losses.^{1,2,6} Evaporative cooling via sweating is by far the most efficient way to remove a large amount of heat from the

**Fig. 6.1.3**

Changes in plasma volume with steady-state exercise. Initial drop in plasma volume (0–3 min) due to fluid shifts, secondary changes due to fluid losses. ○ = intact horses; ● = splenectomized horses. (Reproduced with permission from McKeever et al.¹⁷)

body.^{1,2,4-6,39} But this ability to maintain body temperature comes at a cost to the cardiovascular system. First, a portion of blood flow that could be used to supply the working muscles is redistributed to the skin to transport heat from the core to the surface.⁵⁻⁸ At moderately heavy work intensities limited circulating blood volume and cardiac output cause transient alterations in cardiac filling volume and MAP that are sensed respectively by the cardiopulmonary baroreceptors (volume receptors) and by the high-pressure baroreceptors.⁵⁻⁸ Optimal perfusion of working muscles requires that the cardiovascular system keeps MAP within narrow limits.⁵⁻⁸ However, there is an

upward change in the set point during exercise that removes the check on the system that would tend to limit an increase in cardiac output. Rowell^{7,8} has suggested that a feed-forward mechanism allows blood pressure to increase during exercise via integrated responses of the above-mentioned two layers of defense.^{7,8} Control of MAP during increasing exercise intensity necessitates shunting of blood away from non-obligate tissues.^{6-8,35,36} These non-obligate tissues include the splanchnic and renal vascular beds.^{6-8,35,36,40,41} The changes in vascular tone are so pronounced that they are facilitated by both nervous system and endocrine effector signals.^{6-8,40,41} Interestingly, these neural and endocrine factors (catecholamines, plasma renin activity, vasopressin, etc.) influencing vascular tone in the splanchnic and renal vascular beds increase at intensities above 50–60% $\dot{V}O_{2\text{ max}}$ ^{6-8,29,33,40,42-45} in most cases prior to any substantial loss of sweat. These adjustments in cardiovascular function and renal blood flow thus appear to be a response to the 'stress of exercise' itself rather than fluid and electrolyte imbalance.⁴⁰

As exercise progresses, acute fluid shifts from the vascular compartment to the interstitial space provide water for sweat production.⁶⁻⁸ Sweat loss causes a net reduction in total body water and a decrease in plasma volume that, if not replaced by water intake, eventually results in decreased venous return and cardiac filling pressure.⁶⁻⁸ The horse has evolved to have a large reservoir of fluid in its digestive tract.⁶ This is not an inconsequential amount as the horse can utilize fluid from the large colon as well as fluid contained in its cecum. Thus, in the wild the horse does not need to stop to drink while running from a predator. There are limits to these fluid reserves and the horse produces a huge volume of hypertonic sweat that eventually can lead to a compromised vascular volume. The cardiopulmonary volume receptors sense the drop in cardiac filling pressure and volume. To keep cardiac output constant the resultant fluid loss-induced decrease in stroke volume must be countered by an increase in heart rate.⁶⁻⁸ This tachycardiac adjustment to progressive fluid loss is referred to as cardiovascular drift.⁶⁻⁸ More dramatic reductions in flow to non-obligate tissues can occur if increased heart rate cannot compensate for the decrease in cardiac filling pressure. Sweat-induced reductions in total body water initially come at the expense of plasma volume; however, as exercise progresses, water loss causes a progressive cellular dehydration.^{1,2,4-6,9,39,45} The resultant cellular fluid deficit eventually leads to decreased cell function, fatigue, and a failure to thermoregulate

properly.^{46,47} Other problems can be caused by significant simultaneous electrolyte losses.^{2,4,5,39,45-47}

Along with heavy sweat production comes loss of electrolytes which stimulates a variety of endocrine responses which function to correct the concentration of these vital substances.⁶ Human sweat is hypotonic; that is, the concentration of sodium is less than that found in the plasma.² While prolonged exercise can result in severe electrolyte disturbances recent research has shown that they are much less frequent in humans compared to those seen in horses.^{2,6,45-47} Equine sweat, on the other hand, is hypertonic and excessive sweat loss can rapidly result in severe problems related to both the hypovolemia and to the resulting electrolyte imbalance.^{1,2,39} Several papers have documented that severe electrolyte loss can lead to weakness, muscle cramps, acid-base imbalance, and decreased performance.^{2,5,6,45-47} Interestingly, trained humans appear to start sweating earlier and in greater amounts than untrained individuals exercising at similar relative work intensities.^{6,26,48} Trained human athletes also appear to have a more hypotonic sweat^{11,48} that results from aldosterone's action on the sweat glands.^{19,20,25,26,48} Studies have not yet demonstrated similar training-induced adaptations in the neuroendocrine control of sweating and fluid balance in horses.

Sweat losses and the combined effects of exercise and environment

The transduction of potential energy into kinetic energy is a rather inefficient process with only 20–30% of potential energy effectively utilized for work.⁵ The rest is heat that must be liberated from the body.⁵ In general, the greater the exercise intensity of the event, the greater the heat load generated and the greater the need for heat dissipation.⁵ Even under mild ambient conditions, the exercising horse is presented with a significant thermoregulatory challenge that requires an integrated response to transport heat from the core to be transferred to the environment.⁵ The major method for the transfer of heat from the body involves sweating and evaporative cooling.⁵ Evaporative cooling is several times more effective than other routes of heat exchange. Because evaporative cooling is so essential, the horse appears to have evolved with several species-specific adaptations that enhance the

ability to move fluid from the vascular compartment to the sweat glands and ultimately to the exterior as sweat.⁵ First, as previously mentioned, increases in hydrostatic pressure during exercise enhance fluid shifts from the vascular compartment to the interstitial space, increasing the availability of fluid for sweat production.^{5,6,19,20} Second, the sweat gland of the horse is very simple compared to the well-organized sweat glands seen in humans.^{5,39} Therefore, sweat excretion is a less complex process. Additionally, the equine sweat gland, unlike the human sweat gland, is not responsive to aldosterone and thus it cannot conserve sodium.³⁹ Put simply, the equine sweat gland almost acts like a funnel to allow a hypertonic solution of electrolytes to move from the interstitial space to the surface. Teleologically, producing a hypertonic sweat may be beneficial as the solvent drag would tend to facilitate the movement of a greater amount of water outward. The extra salt in the sweat, as well as the protein latherin, also alters the evaporation point of the sweat, possibly enhancing evaporative cooling. Functionally, this is significant as the horse has a less favorable surface area to volume ratio when compared to humans. The down side to these adaptations is the potential for large fluid and electrolyte deficits.

Recent articles have reported that during submaximal exercise, under conditions of high heat and humidity, sweat losses in horses can exceed rates of 12 L per hour.^{5,39,45,49,50–56} This large volume of sweat results in proportional decreases in bodyweight, total body water, and plasma volume. This, in turn, can compromise venous return, cardiac filling pressure, cardiac output, and the ability to thermoregulate. Thermoregulatory stability requires a large cardiac output and peripheral blood flow to carry heat from the core of the body to blood vessels in the skin.⁵ At the same time, the heart must pump blood to the working muscles, to the brain, and to other 'obligate' tissues that cannot suffer from reduced perfusion. To maintain cardiac output during intense exercise, baroreflexes cause selective vasoconstriction and blood flow redistribution.^{6–8} This reduces blood flow to non-obligate tissue beds like the viscera and kidneys and allows for increased blood flow to the working muscles.^{7,8} As core heat accumulates, various feedback mechanisms cause blood flow to increase to the skin to enhance the transport of heat from deep in the core of the body to the surface.⁵ If exercise proceeds for a long enough time, sweat loss leads to progressive dehydration and loss of plasma water from the bloodstream. Dehydration causes a decrease in circulating blood volume and cardiac stroke volume. A horse can

keep going despite this reduction in vascular fluid volume; however, to maintain cardiac output, heart rate must increase ('cardiovascular drift').^{5–8} When dehydration cannot be compensated for by cardiovascular adjustments, body temperature rises and is soon followed by decreased performance and fatigue. Maintaining cardiac output and MAP is vital to keeping perfusion pressure at the level needed to distribute flow to the working muscles, skin, and other obligate tissues. Thus, at the onset of exercise both mean arterial blood pressure and skin blood flow are defended.^{5–8,19} However, as a horse becomes dehydrated, MAP is defended preferentially at expense of skin blood flow and thermoregulation, adding to the resulting increase in body temperature.^{5–8,19}

Several laboratories have observed that, even under cool conditions, endurance exercise performed in the field or on a treadmill laboratory will cause a substantial rise in core temperature, a substantial amount of sweat production, and a dramatic loss of total body water.^{1,2,5,39,45,49,50–56} In field trials, it has been documented that even with the combination of proper hydration, adequate sweating, and maximal rates of evaporative cooling, a horse's body temperature can reach 105–106°F during endurance rides performed under moderate climatic conditions.^{1,2,5,39,45,49,50–56} Under hot humid conditions, evaporative cooling becomes ineffective because sweat will not evaporate. The resulting hyperthermia can cause fatigue, cramps, heat stroke, and other thermal injuries.^{1,4–6,10} Thus, in a hot and humid environment, even a well-hydrated horse can encounter potential life-threatening situations in a relatively short time. However, most of these injuries can be prevented with proper feeding, adequate watering, and advanced planning of exercise training sessions and athletic events.

Almost all studies reporting sweat electrolyte concentrations in horses during exercise demonstrate that the horse loses a large amount of key electrolytes.^{1,2,5,39,45,49,50–56} The range of sweat electrolyte concentrations varies, with older studies suggesting tremendous sodium and chloride losses.² The magnitude of electrolyte loss reported in some studies is questionable and most likely is a function of the methodologies utilized. Older studies relied on sweat scrapings and other sampling techniques that overestimate actual losses. Several newer papers have utilized more refined methods adapted from human research, preventing the errors due to evaporation of water or addition of salt from areas already contaminated by prior sweating.^{49,50–56} Nevertheless, more recent studies still demonstrate that equine sweat is hypertonic to plasma

and that without replacement there are substantial electrolyte deficits in horses competing in endurance activities. While sodium and chloride are the primary electrolytes lost in equine sweat, other key electrolytes like magnesium and calcium are also lost.³⁹ Most important is a disproportional loss of chloride ions that can potentially lead to a serious metabolic alkalosis.^{9,39} The loss of sodium can become an even greater problem during recovery if a horse drinks too much water.⁹ As with human marathon runners, some endurance horses can develop a hyponatremia that, if untreated, can lead to collapse and death.⁴⁷ Thus, provision of water as well as electrolyte supplements is warranted after endurance activities accompanied by large fluid and electrolyte losses.

Thirst, drinking, and electrolyte intake

Hormonal changes stimulate thirst and drinking and it is well recognized that the horse has a finely tuned regulatory system to maintain fluid and electrolyte balance.^{1,2,6,19} Mechanistically, thirst can be stimulated by increases in circulating concentrations of angiotensin and arginine vasopressin (AVP), and by changes in the concentration of calcium and other electrolytes in the cerebrospinal fluid.^{6,24–26,28,57–60} These systems are modulated by changes in osmolality sensed by the paraventricular and supraoptic nuclei of the hypothalamus.^{6,24–26,28,57–60} Thus, a small increase in plasma osmolality will result in the release of AVP by the posterior pituitary.^{6,24–26,28,57–60} These drives for thirst and drinking are finely tuned for the resting horse; however, it has been documented that strenuous, high-intensity exercise can paradoxically suppress thirst and the central drive for drinking behavior in humans, dogs, and more recently horses.^{6,24–26,28,57–60} Mechanistically, it appears that early in exercise there is a suppression of AVP release associated with the role the cardiopulmonary baroreceptors play in the accommodation of fluid shifts and the mobilization of the splenic reserve.^{19,61} Comparative studies of dogs, humans, and other species have also shown that there is a general suppression of the drive for drinking, as well as additional suppression of thirst and drinking during exercise when cold hypotonic water passes by nerves in the mouth and throat.^{6,24–26,28,57–60} This suppression of drinking behavior may be a protective mechanism — an ingrained defensive behavior that prevents an indi-

vidual from stopping for water while on the run from a predator. Horse owners and veterinarians monitoring endurance events have reported a similar suppression of drinking in horses.^{6,9,10,23} In many cases, endurance horses will not drink at the end of a race and some clinicians have reported that this suppression of thirst and drinking behavior can last for several hours after exercise.^{1,2,10,23} Unfortunately, this is the time when rehydration should be taking place. In some cases, it is the horse with the most severe fluid and electrolyte deficit that is the one that will not drink right away or is the one that will consume a volume that is not sufficient to replace the amount of water lost during competition.^{10,23} Some researchers have speculated that there is a threshold where severe dehydration itself accentuates this paradoxical suppression of drinking.^{26,28,57–60} Interestingly, recent research demonstrated that endurance horses could be taught to drink warm water with electrolytes during competition without a resulting suppression of thirst and drinking.^{62–66}

An equine athlete can usually recover from moderate acute exercise-induced fluid and electrolyte losses. And, in most cases, fluid and electrolyte losses following acute exercise can be compensated for through the provision of adequate water, a normal diet, and a salt and mineral supplement.^{10,67} However, some scenarios can potentially cause problems for competing animals.^{10,67} One scenario might result from larger-than-normal fluid and electrolyte losses resulting from exercise in hot or hot and humid conditions. In this case, fluid losses may need to be replaced rapidly to avoid thermal injuries.¹⁰ Competition following long periods of trailering to an event with a limited amount of water supplied for drinking while in transit presents another scenario for problems.¹⁰ In this case, a horse owner should provide access to water prior to competition.¹⁰ If the distance to the event is long, one should consider providing opportunities for drinking at rest stops along the way.¹⁰ Another situation that can result in severe dehydration can occur when there is limited recovery time between phases during three-day competitions or other multiple event competitive formats.¹⁰ Logistics of these events may prevent an animal from drinking enough water or prevent a horse from getting adequate electrolytes via dietary replacement.¹⁰ Clinicians monitoring the status of competing horses should insure that all animals have had an ample opportunity to recover between days, phases, or heats of a competition.¹⁰

The amount of water consumed by a horse varies with the individual, diet, climate, and amount of exer-

cise.^{10,66} The National Research Council guidelines for feeding horses recommend 2–4 L of water per kilogram of dry matter feed intake.^{10,67} This amount can increase 15–20% for horses in warm environments.^{10,66} For example, a recent paper reported that non-exercised horses in Arizona consumed between 30 and 40 L of water in the hot months of June and July.¹⁴ Water intake may increase 300% or more with prolonged exercise and some studies have documented that an active horse can consume 100 L of water per day under some conditions.^{1,2,39,62}

Renal function during exercise

Alterations in renal blood flow and renal function vary with both the duration and the intensity of exercise.^{6,40,68–70} In general, exercise affects both renal blood flow and the delivery of water and electrolytes to the kidney, and it affects mechanisms associated with the tubular reabsorption of water and electrolytes.^{6,40} Acute and chronic renal responses to exercise are part of an integrative defense of blood volume, blood pressure, and osmolality.^{6,40} Zambraski⁴⁰ suggests that the alterations in renal function are both a response to the stress of exercise alone and also to perturbations in fluid and electrolyte balance (Fig. 6.1.4). Acute demands of exercise result in a decreased renal blood flow, sparing cardiac output so as to allow the cardiovascular system to meet the increased circulatory demand associated with exertion.⁴⁰ Post-exertional changes in renal function, on the other hand, are part of a long-term mechanism to restore lost water and electrolytes.^{6,40} The kidneys also function as major effector organs in the adaptive expansion of plasma volume and electrolyte content balance referred to as the hypervolemic response to exercise training.^{6,40}

Effects of exercise on renal blood flow

During submaximal exercise absolute renal blood flow (RBF) is not reduced in humans or horses.^{40,71} However, it has been documented that low-intensity exercise results in a reduction of RBF as a percentage of cardiac output.^{40,71} For example, Hinchcliff et al⁷¹ reported that RBF averaged 15 mL/kg/min and did not change during exercise. However, because cardiac output increased, RBF did decrease as a percentage of cardiac

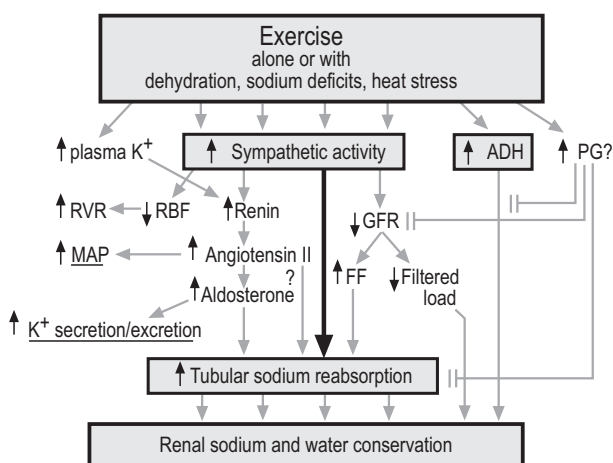


Fig. 6.1.4

Renal response to high-intensity exercise. ADH, antidiuretic hormone; FF, filtration fraction; GFR, glomerular filtration rate; MAP, mean arterial pressure; PG, prostaglandins; RBF, renal blood flow; RVR, renal vascular resistance. (Reproduced with permission from Cooper Publishing Group, Traverse City, MI.⁴⁰)

output, dropping from 23% at rest to 6% during exercise.⁷¹ High-intensity exercise, on the other hand, causes substantial reductions in absolute renal blood flow in swine, horses, and humans, but not in normal dogs.^{6,40,72,73} Renal vasoconstriction appears to occur when work intensities exceed a threshold of 50–60% of $\dot{V}O_{2\max}$,^{6,40} a point coincident with detectable increases in renal nerve activity, circulating catecholamines, and plasma renin activity.^{6,40–42}

Schott and co-workers⁷² were the first to demonstrate that high-intensity exertion causes a reduction in both absolute RBF and relative RBF (i.e. RBF expressed as a percentage of cardiac output) in the horse. Absolute RBF decreased from 9.0 L/min to 2.4 L/min when horses were exercised at a speed shown to produce an oxygen uptake that was 100% of $\dot{V}O_{2\max}$.⁷² Amazingly, RBF decreased as a percentage of cardiac output from 22% at rest down to 0.09% during maximal exercise.⁷² This reduction in RBF resulted in a substantial drop in glomerular filtration rate and subsequently a drop in urine flow, and the excretion of solute-free water and various electrolytes. Interestingly, a follow-up study demonstrated that phenylbutazone and furosemide did not appear to alter the renal response to high-intensity exercise.⁷³

Effect of exercise on glomerular filtration rate, filtration fraction

Blood flowing through the renal artery is filtered through millions of glomeruli in the kidney.⁴⁰ The glomerulus is part of the nephron, the basic structural unit of the kidney.⁴⁰ It is a complex structure made up of the afferent artery, Bowman's capsule, and the efferent artery.⁴⁰ Algebraically, glomerular filtration rate (GFR) is representative of the sum of the action of all the nephrons, and is autoregulated over a wide range of kidney blood flow. As with RBF, the effects of exercise on GFR in the horse varies with the intensity of the exercise.^{71–73} GFR has been shown to increase or decrease during submaximal exercise depending on hydration status.⁴⁰ In studies where the subjects were hyperhydrated, GFR did not change during submaximal exertion.⁴⁰ However, when individuals were euhydrated or hypohydrated, changes in GRF were intensity- and/or duration-dependent, changing the most when individuals performed high-intensity exercise.⁴⁰ Interestingly, while RBF can be dramatically reduced during exercise, studies of humans show that decreases in GFR do not necessarily parallel the decreases in RBF.⁴⁰ Thus, glomerular filtration is somewhat protected in the face of exercise-induced reductions in RBF with a resultant increase in filtration fraction (i.e. the ratio GFR/RBF).⁴⁰

Zambraski⁴⁰ has suggested some possible mechanisms to explain the exercise-induced increase in filtration fraction (FF) observed in humans and other species: first, maintenance of glomerular hydrostatic pressure and preservation of GFR through greater constriction of the efferent arteriole relative to the afferent arteriole.⁴⁰ However, data from comparative species are lacking or have not fully supported this hypothesis. A second hypothesis for exercise-induced increases in FF postulated that changes in glomerular capillary Kf simultaneous to decreases in glomerular capillary hydrostatic pressure would 'assist in maintaining GFR'.⁴⁰ While not directly tested, this mechanism fits teleologically with data from human and animal studies documenting exercise-induced increases in substances that affect Kf, such as vasopressin, the prostaglandins, and angiotensin II.⁴⁰

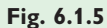
The effects of exercise on GFR and FF appear to be similar in the exercising horse and in exercising humans. Hinchcliff and co-workers⁷¹ reported that, as in humans, there appear to be no alterations in GFR or FF during standing control or submaximal (50–60% $\dot{V}O_{2\max}$) exercise in horses. In that study, creati-

nine clearance (GFR) ranged between 2.0 and 2.5 mL/min/kg and FF averaged 23%.⁷¹ High-intensity exercise, on the other hand, produces a significant decrease in GFR and a significant increase in FF in the horse.⁷² Schott and co-workers⁷² demonstrated that GFR decreased 73% from a mean of 1.9 mL/kg/min to 0.5 mL/kg/min during exercise performed at an intensity shown to produce $\dot{V}O_{2\max}$. As with humans, horses performing high-intensity exercise had significant increases in FF from 16% at rest to 23.2% following running.⁷² While drugs like furosemide and phenylbutazone affect renal blood flow, they do not appear to alter GFR and FF in the horse during submaximal or maximal exercise.⁷³ These observations were interpreted to suggest that the renal prostaglandins play a minimal role in mediating changes in GFR and RBF in the horse during exercise.⁷³

Renal tubular function and excretion during exercise

In simple terms, the kidneys filter the blood at the glomerulus and then selectively reabsorb or secrete essential and non-essential substances in the tubules.^{6,40} Normal fluid and electrolyte homeostasis requires the kidneys to eliminate excess water and electrolytes, or if there is a deficit, to reabsorb those vital components of the blood.^{6,40} Alterations in GFR and/or tubular handling of water and solutes ultimately affects the volume of urine produced and the rate volume of essential electrolytes excreted over a given period of time.^{6,40} Studies of humans, dogs, and horses have demonstrated that changes in tubular handling of water and solutes varies with work intensity.^{6,40} These changes appear to be secondary to alterations in renal blood flow, GFR, and the filtered load of a given substance.^{6,40} We are aware of only a few studies of the effects of exertion on renal tubular function. One study (Fig. 6.1.5) examined the effects of 1 h of submaximal exercise on endocrine changes and renal tubular function⁷⁴ and the others examined the effects of high-intensity exercise on renal function during and after exercise.⁷²

During submaximal exercise, performed at an intensity below 60% $\dot{V}O_{2\max}$, urine flow increases in humans and horses.^{40,69,71} However, in horses, while low-intensity exercise resulted in a significant 45% increase in urine production, the total volume of extra water lost (~6 mL/min) was reported to be small compared with the increased volume lost as sweat.⁷²



McKeever et al⁷⁴ reported that the increase in urine flow was due to an exercise-induced increase in osmotic clearance induced primarily by a natriuresis and a kaliuresis.⁷⁴ The increase in sodium excretion appeared to be mediated by a concomitant increase in plasma atrial natriuretic peptide (ANP).⁷⁴ Even so, the total amount of sodium lost via renal excretion was minimal. The authors suggested that, during exercise, ANP (a potent vasodilator) functioned primarily to facilitate decreased vascular resistance in the working muscles⁷⁴ so as to accommodate increased atrial pres-

Interestingly, the authors of that same study demonstrated that there was a significant kaliuresis as well as the aforementioned natriuresis and suggested that the increase in potassium excretion was due primarily to a rise in plasma aldosterone concentration.⁷⁴ The

increase in plasma K^+ concentration seen in submaximally exercised horses coincided with an increase in plasma aldosterone concentration.⁷⁴ The authors speculated that because there were limited decreases in plasma Na^+ concentration, the increases in aldosterone release may have been primarily in response to the rise in plasma K^+ concentration.⁷⁴ This phenomenon has been demonstrated by other researchers, who have shown that the most potent stimulus for aldosterone secretion is an increase in circulating potassium.⁷⁴ Functionally, this prevents excessive increases in plasma K^+ concentration, which can alter electrophysiological gradients in the muscles and other tissues.⁷⁴ Several studies have documented that excessive increases in plasma K^+ concentration appear to be one of many factors contributing to the onset of muscle fatigue.^{6,74} Thus, the reported increase in plasma aldosterone release and the kaliuretic action of ANP may function to limit an excessive rise in plasma K^+ concentration during lower-intensity exercise.⁷⁴

Another major problem associated with exercise in the horse is an excessive and disproportional loss of chloride via the sweat.⁷⁴ McKeever et al⁷⁴ demonstrated that renal chloride losses decrease when plasma chloride concentrations fall. Similar reductions in chloride excretion are also seen in exercising humans.^{6,40} As Na^+ and K^+ excretion increased in submaximally exercised horses, the authors suggested that mechanisms affecting the conservation of those electrolytes could not have been responsible for the increase in Cl^- reabsorption.⁷⁴ Interestingly, the same horses became alkalotic during the hour-long bout of exercise.⁷⁴ Thus, the authors suggested that based on other reports,^{40,74} renal mechanisms affecting reabsorption of Cl^- and secretion of HCO_3^- by the antiporter in the apical membrane of the intercalated cell of the cortical collecting duct may have led to conservation of chloride.⁴⁰

Lastly, solute-free water clearance does not appear to be altered by submaximal exercise in the horse despite significant increases in plasma osmolality and plasma vasopressin concentrations.⁷⁴ Similar observations have been made in exercising humans.⁴⁰ This paradox has several possible explanations. First, a decrease in renal blood flow potentially could decrease the filtered load of solute-free water available for reabsorption by the action of AVP on the collecting ducts.⁴⁰ This explanation would not be supported by the observation that submaximal exercise does not affect absolute RBF or GFR.^{6,71} It may, however, be

possible that the extrarenal functions of vasopressin are more important during exercise and actions on the kidneys are overridden. Such functions may include vasopressin's role in vasoconstriction of vascular beds in non-obligate tissues, its role in central mechanisms that stimulate thirst and drinking, and its action on the gut.^{6,25,75,76} In the gut, vasopressin appears to act on the epithelium of the large intestine enhancing the uptake of sodium and water.⁷⁵ This protective effect would aid in the limitation of exercise-related fluid deficits more than potential reductions in free water clearance.

High-intensity exercise appears to have effects that are dramatically different from submaximal exertion. Sodium excretion is dramatically reduced during high-intensity exercise in horses, pigs, and humans.^{6,40,71} Inconsistent changes in sodium excretion have been observed in the exercising dog.⁴⁰ Several mechanisms could be responsible for the decrease in sodium excretion including:

- a decrease in filtered load of sodium
- activation of the renin–angiotensin cascade
- elevation of plasma aldosterone concentration
- direct neurogenic control.⁴⁰

In the first instance, a change in filtered load sodium would mean that less solute would be presented to the tubules for reabsorption. This would require a reduction in GFR; as this does not change in humans, pigs, or horses,^{6,40,71} it does not appear to be a viable mechanism for the decrease in sodium excretion. More recent information has been published that shows that the decrease in sodium excretion is not blocked by pharmacological blockade of the renin–angiotensin cascade.⁴⁰ Aldosterone concentration increases in the horse without a change in sodium excretion.⁷⁴ In other species, the observation that sodium excretion rapidly returns to baseline after exercise suggested that aldosterone was not the mediator of the antinatriuresis seen during exercise.⁴⁰ The speed of the recovery has been further interpreted to suggest a neural mechanism.⁴⁰ This theory is consistent with a reported intensity-dependent increase in renal sympathetic nerve activity during exercise.⁴⁰ Zambraski⁴⁰ and others have suggested that, based on all the current evidence, the mechanism behind exercise-induced increases in sodium reabsorption is primarily direct neurogenic control.

Schott et al^{72,73} reported that urine flow almost stopped during supramaximal exercise and was still

below pre-exercise levels in the period immediately after exercise. High-intensity exercise also caused a decrease in urine osmolality and osmotic excretion.^{72,73} Interestingly, one would have predicted a significant reduction in electrolyte excretion; however, there were only non-significant reductions in the bulk excretion of K^+ and Cl^- during exercise and no change in Na^+ excretion, despite a reduction in urine flow.⁷² This response contrasts with changes observed in other species.⁴⁰ One explanation for this aberrational finding may be the design of the experiment. In the experiment, exercise was performed during part of one of the 15-min collection periods.⁷² Data for the entire 15-min collection period were pooled and included a post-exercise period characterized by diuresis, natriuresis, and kaliuresis (see below). These post-exercise changes may have offset any exercise-induced decreases in electrolyte excretion that should have occurred with a reduction in RBF and urine flow.

High-intensity exercise appears also to affect urinary pH, an observation of interest to racing chemists.⁷⁷ Gerken et al⁷⁷ reported that high-intensity exercise caused a transient reduction in urine pH that lasted for up to 60 min of recovery. The authors suggested that the more acidic urine may affect the results of the battery of tests used to detect foreign substances.⁷⁷ More interestingly, they suggested that alkalizing agents like sodium bicarbonate may alter post-exertion pH, thus further complicating drug detection efforts.

Post-exercise changes in renal function

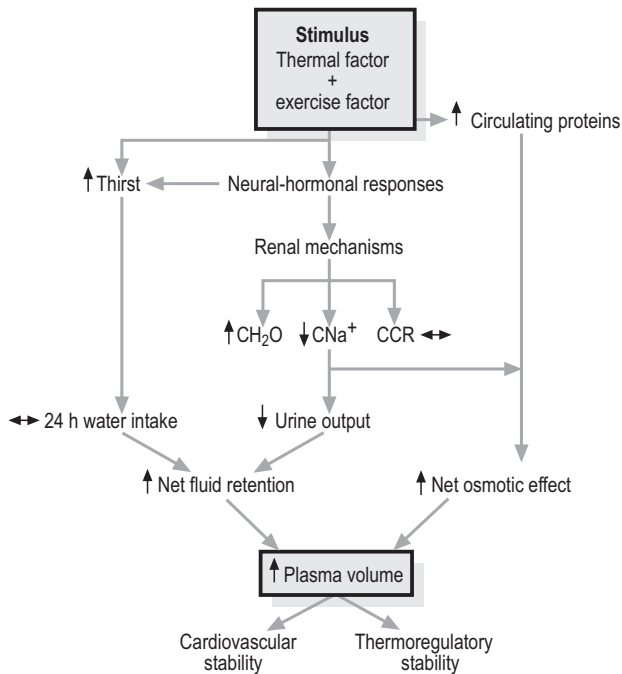
While the studies of Schott et al^{72,73} did not document a change in tubular function, the authors did report a substantial post-exercise increase in urine flow. Data were consistent with a diuresis, kaliuresis, and natriuresis.^{72,73} Excretion rates for these substances returned to baseline by 30 min of recovery. The authors suggested that the increase in sodium and potassium excretion was most likely due to an increase in atrial natriuretic peptide, which had been shown to increase during exercise in the horse.⁷² Long-term, the adaptations to exercise training involves reduction in 24 h urine output and an expansion of plasma volume with a concomitant increase in the content of sodium in the vascular compartment.^{14,78}

Adaptive response to repeated exercise (training)

Training-induced hypervolemia

Repeated exercise or training usually evokes an adaptive response that better prepares the horse's various physiological systems for subsequent bouts of acute exertion.^{6,11,14,48,79} Disturbances in fluid and electrolyte balance require a two-phase response, with the early phase resulting in the replenishment of acute fluid and electrolyte losses and a secondary or adaptive phase that results in an enhanced ability to cope with future systemic disturbances.^{6,11,14,48} This 'hypervolemic' response to training is an adaptive response that involves a beneficial increase in blood volume due to an increase in plasma volume.⁴⁸ The training-induced hypervolemia is beneficial because it enhances both cardiovascular and thermoregulatory stability during the challenge of acute exercise.^{48,80} The increase in total body water provides extra fluid that insures cardiovascular stability by providing the extra volume needed to maintain venous return and thus cardiac output.^{6,48} Thermoregulatory benefits are two-fold, including an increase in the ability to increase skin blood flow to enhance transport of heat from the core to the surface and an increase in the amount of fluid available for sweat production and evaporative cooling.⁴⁸ Functional evidence for the latter benefit can be seen in studies of humans that have demonstrated that trained individuals have an earlier onset to sweating and produce more sweat compared to untrained individuals exercising at the same relative submaximal work intensity.⁴⁸ It is likely that there are similar training-induced changes in cardiovascular function and thermoregulation in the horse.⁸⁰

Mechanistically, the retention of water and electrolytes that leads to a training-induced hypervolemia reflects the effort of multiple systems to defend volume, plasma osmolality, and blood pressure.^{6,11,14,19,48} Comparative studies have demonstrated that approximately 60% of the mechanism behind the hypervolemic response is related to stimuli associated with the demands of thermoregulation.⁴⁸ The remaining 40% of the response appears to be related to mechanisms directly associated with exertion.⁴⁸ These mechanisms counter acute fluid and electrolyte losses by stimulating the intake of water and by reducing renal losses of water and electrolytes (Fig. 6.1.6).

**Fig. 6.1.6**

Suggested mechanisms for the exercise training-induced hypervolemia seen in horses. CCR, creatinine clearance. (Adapted from Convertino.⁴⁸)

Interestingly, rats, humans, dogs, and horses all expand their plasma volume in response to exercise training.^{14,48,79,81} However, there are profound species differences in the mechanism behind the hypervolemic response to exercise training. Dogs expand their total body water through drinking; in fact, water is consumed at a greater rate than an observed training-induced natriuresis and diuresis.⁸¹ Cooling in dogs involves a large loss of respiratory water and saliva, which results in acute increases in plasma tonicity and osmolality.⁸¹ Thus, dogs must either drink to take in water to dilute the hyperosmotic hypertonic plasma or they must use renal mechanisms to excrete the excess electrolytes sensed in the plasma.⁸¹ Humans and horses, on the other hand, lose a large amount of electrolytes in their sweat; therefore, there is a drive to replenish fluid and electrolytes to defend both volume and tonicity.^{14,48,78}

In horses and humans, drinking during and immediately after exercise at best only slows or partially counters the development of a fluid deficit, but does not counter any electrolyte deficit.^{14,48,78} Studies of horses and humans are mixed as to the role of water and electrolyte intake in the long-term response to exercise training.^{14,48,78} Humans use both an increase

in thirst and drinking, and renal mechanisms to increase net fluid retention.⁴⁸ Drinking in humans, however, does not account for all of the net water retention, with most of the actual expansion of total body water coming through renal mechanisms.⁴⁸ One recent study of the horse reported that water intake increased with training; however, the authors did not measure renal losses or conduct a balance study that would determine if the amount ingested contributed to an expansion of plasma volume.⁶ Other studies have shown that exercise training does not alter water intake in the horse.^{14,78} Instead, they reported that the horse appears to rely on renal mechanisms and an overall reduction in urine water loss^{14,78} to retain the sodium and water needed to expand plasma and blood volume.

In both humans and horses, this decrease in urine output seen with training is due to alterations in post-glomerular mechanisms rather than a change in filtration rate.^{14,48,78} These renal adjustments are an adaptive or training response rather than a countermeasure to the perturbations of acute exercise. In humans an aldosterone-mediated retention of sodium and water seems to cause a net retention of water.^{82–84} Until recently, the mechanism behind the hypervolemic response was not as clear in the horse. An early study did not demonstrate any change in renal mechanisms affecting the retention of sodium and water, and instead suggested that urea rather than sodium may be the solute responsible for the renal retention of water that leads to a training-induced expansion of plasma volume in the horse.¹⁴ However, the authors paradoxically demonstrated that there was a highly significant increase in plasma sodium content that paralleled the increase in plasma volume.¹⁴ Interestingly, this net increase in retained sodium and water occurred despite increased losses via sweating.¹⁴ As the rate of sodium intake was held constant, the only other routes for a training-induced retention of sodium would have been either a more efficient uptake of electrolytes and water from the gut and/or a net retention by the kidneys early in the first days of training, as seen in humans, where most of the response occurs in the first days of training. To solve this mystery, a more recent paper⁷⁸ focused on the first days of training and demonstrated dramatic reductions in urine output and excretion of sodium during the first days of training. Thus, like humans, the horse appears to undergo a similar aldosterone-mediated retention of sodium and water by the kidneys.⁷⁸

However, it was also found that renal retention of sodium and water did not fully counter losses seen in

the sweat during the first days of training and the authors suggested that an enhanced aldosterone-mediated uptake of sodium and water from the large intestine may also contribute to the retention of electrolytes and water.⁷⁸ This makes sense because the horse's large intestine serves as a fluid reservoir. Such an additional response in the horse may be a warranted species-specific adaptation in response to the relatively larger electrolyte deficits associated with the production of hypertonic sweat. Enhanced intestinal uptake of sodium and water is supported by other published studies⁸⁵ demonstrating that aldosterone may enhance the transport of electrolytes and water from the digestive tract of the horse.

Concurrent with the aforementioned retention of water, sodium, and other vital electrolytes (which keeps the retained fluid isotonic) is an increase in the plasma protein content.^{14,48,78} This increase in protein functions to keep the plasma iso-oncotic, thus holding water within the vascular space. Human studies suggest that the early increase in plasma protein content comes about from inward shifts of protein from the lymphatics and interstitial space, and later from an overall net increase in plasma protein synthesis.⁴⁸ The hypervolemic response to training in the horse also appears to involve an increase in the content of plasma protein, most likely through a net increase in synthesis.^{14,78}

One aspect of the effect of training yet to be studied in the horse is whether there are alterations in the cardiopulmonary baroreceptors that allow for the retention of the extra vascular volume. Human studies have shown that training results in a downregulation of the ANP and neuroendocrine response to exercise, quite possibly to accommodate the hypervolemia associated with exercise training.⁴⁸ Future research should determine if downregulation of these important control mechanisms also occurs in horses.

It is important to note that short-term horizontal studies of humans, dogs, and horses have demonstrated that the increase in plasma volume occurs early in training and is followed by a subsequent slow increase in red blood cell volume (Fig. 6.1.7).⁸⁶ Thus, early in training, if one only looks at hematocrit there is a false impression of a 'sports anemia'.⁸⁶ Interestingly, in humans there appears to be an overshoot in the expansion of plasma volume.⁸⁶ As red cell volume slowly increases, the early increases in plasma volume appear to level off and even decrease.⁸⁶ Thus, after weeks and months of training there is a greater blood volume, with both plasma and red cell volume remaining greater than pre-training levels,⁸⁶ most likely at a

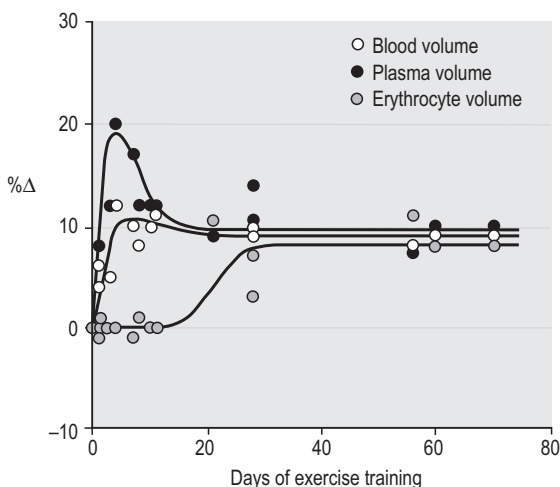


Fig. 6.1.7

Estimated time course of relative (%) changes in blood volume, plasma volume, and erythrocyte volume during exercise. Each point represents the average change reported in a group of (human) subjects from one investigation. Data were extracted from 18 investigations in which all three vascular volumes were reported. (Reproduced with permission from Sawka et al.⁸⁶)

level that optimizes both blood viscosity and oxygen-carrying capacity.⁸⁷

Effects of aging on the acute and chronic response to exercise

A limited number of data have been reported comparing the thermoregulatory responses of older and younger men and women during exercise in the heat.^{88,89} It has been concluded that age influences thermoregulatory function during exercise.⁸⁸ Suggested reasons for this age-related decline in the ability to thermoregulate properly during exercise in humans include lower cardiovascular capacity due to the age-related decrease in cardiac output, alterations in mechanisms associated with the control of skin blood flow, and a possible state of hypohydration in the elderly.⁸⁸

While there is an abundance of papers that have examined thermoregulation in young horses, few studies have addressed the effects of age on the thermoregulatory response to exercise in the horse.^{90,91} McKeever and co-workers^{90,91} exercised young and old

horses at the same submaximal absolute work intensity of 1625 watts until they reached a core body temperature of 40°C. Older horses reached a core temperature of 40°C in almost half the time required by the younger mares.^{90,91} The heart rates of the older mares were also substantially higher than the heart rates of the younger mares at 40°C.^{90,91} Interestingly, both groups had similar heart rates and core temperatures by 10 min after exercise.^{90,91} Even with the more rapid heart rate, older horses were still unable to dissipate the heat generated from exercise as quickly as younger mares, therefore leading to a faster increase in core temperature after the onset of exercise. Age-related changes in fluid and electrolyte balance and cardiovascular function may contribute to the impaired thermoregulatory capacity. Older humans commonly have lower total body water, plasma volume, and reserves of fluid for sweating.⁸⁸ In the above mentioned studies of aged horses the changes in markers of fluid status suggested that acute fluid shifts were of a similar magnitude when compared to younger animals. However, a subsequent study⁹² demonstrated that older horses had a substantially lower

pre-exercise plasma volume compared to younger animals. A lower plasma volume could result in lower venous return, stroke volume, and cardiac output, and a compromise of thermoregulatory stability.

Summary

Exercise places large demands on the cardiovascular system, and is further complicated by environmental factors. Performance is limited in many respects by fluid and electrolyte stores and the ability to maintain cardiovascular and thermoregulatory stability in the face of severe sweat losses. Studies of the exercising horse have been primarily descriptive and/or associative with only a limited number seeking to identify physiological mechanisms associated with the control of fluid and electrolyte balance. More mechanistic studies are needed to understand fully the integration of the cardiovascular, endocrine, and renal systems in the defense of plasma osmolality, blood volume, and blood pressure.

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Muscle and blood acid–base physiology during exercise and in response to training

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Introduction

The aim of this chapter is to provide an introduction to acid–base assessment in clinically normal horses at rest and performing exercise of different intensities and durations. The physicochemical approach to acid–base assessment will be introduced and used to exemplify the origins of acid–base disturbances during exercise. Also, the impact of diet, alkalinizing agents, furosemide, and selected clinical conditions on acid–base status will be explored briefly. An update on acidosis and skeletal muscle fatigue will be provided and it is hoped that this will help to dispel some of the myths and confusion surrounding lactate, H^+ , and muscle fatigue. At the outset, it is also important to dispel another myth. We often read that plasma and intracellular pH are maintained within narrow limits. This contention is not supported by the research literature in any animal so far studied, including horses. In clinically normal humans and horses, plasma pH can vary from 7.0 to 7.6, although the norm lies close to 7.4. The large range of change in pH represents a four-fold change in $[H^+]$, specifically 25 to 100 nEq/L, with normal plasma $[H^+]$ of 40 nmol/L. While this range of plasma $[H^+]$ can be tolerated, it is true that such changes are eventually accompanied by the activation of mechanisms that return $[H^+]$ back towards 40 nEq/L. Let us now consider reasons for the changes in acid–base status in the horse at rest and during exercise.

Overview of acid–base responses to exercise

Moderate- to high-intensity muscular exercise results in acidification of muscles and blood. The acidification that occurs primarily results from the generation of protons (H^+) within contracting skeletal muscle. The protons are generated from a series of biochemical and physicochemical reactions associated with increased rates of anaerobic energy production. These proton-generating reactions will be detailed below. The protons that are generated continue to be involved in a series of chemical reactions involving carbonic anhydrases and membrane transport proteins that results in the net movement of acid equivalents out of the contracting muscle cells into the interstitium, and from there into the lymphatic system and venous capillary circulation. It is this large and rapid efflux of acid equivalents from contracting muscle that produces the systemic metabolic acidosis associated with moderate- to high-intensity exercise.

In the exercising horse, whole-body acid–base balance is dependent on the integrated responses of the muscular, respiratory, vascular, hepatic, cutaneous, and renal systems. The muscular system, in addition to providing the locomotory force requirement for activity, generates considerable amounts of acid equivalents, resulting in acidification of the intracellular and extracellular fluid compartments. Non-contracting skeletal muscle also provides the largest

tissue mass within the body for the removal of lactate and acid equivalents during high-intensity exercise and the initial recovery period. The respiratory system plays a key role in eliminating acid equivalents as CO_2 at the lung, in addition to extracting the O_2 needed to fuel aerobic cellular metabolism. The vascular system plays an integral role in the transport and distribution of acid and base equivalents throughout the body; this system provides for the ‘buffering’ of the acid–base disturbance by distributing acid equivalents from acid generation sites (contracting skeletal muscle) to other sites (non-contracting skeletal muscle and other tissues). Within the vascular system itself, bicarbonate, plasma proteins, and hemoglobin within red blood cells are also involved in the transport and temporary storage (buffering) of acid equivalents. The hepatic system is a major tissue mass involved in the removal of lactate from the vascular system, thereby removing acid equivalents from the circulation. The cutaneous system is heavily involved in the production and secretion of sweat to the surface of the skin during and immediately following moderate- to high-intensity exercise. Sweat contains large amounts of Na^+ , K^+ , and Cl^- , and different rates of excretion of each ion affect acid–base state of blood leaving the skin. The kidneys are capable of excreting H^+ and lactate at greatly elevated rates during recovery from high-intensity exercise, aiding in the process of recovery from the acidosis of exercise.

Each of the systems described above is capable of modifying the water, electrolyte, and acid–base composition of the extracellular (blood plasma, lymph, interstitial fluids) and intracellular fluid compartments. It must therefore be appreciated that the acid–base status of the blood depends greatly on where and when the blood is sampled. Blood draining intensely contracting skeletal muscle has very high concentrations of H^+ , lactate, K^+ , and CO_2 , whereas blood that drains relatively inactive tissues (jugular venous blood, for example) has markedly lower concentrations of these metabolites and ions; arterial blood is intermediate in composition. Also, the magnitude of change is proportional to the intensity and duration of exercise, and the concentrations of these and other substances change with time of exercise and recovery.

Why is acid–base balance important? A detailed analysis of acid–base balance provides a biochemical and physicochemical description of the state of the organism, or of individual organs and tissues within the body. Furthermore, severe acid–base disturbances are often associated with high-intensity exercise, with prolonged-duration exercise, and with many patholo-

gies. Therefore an understanding of the origins of acid–base disturbances is of interest to both basic and clinical physiologists. Within the context of the present chapter, exercise physiologists remain keenly interested in acid–base balance because of a close association between acidification and muscle fatigue.^{1,2} Considerable research over the past century has identified many effects of increased $[\text{H}^+]$ within skeletal muscle (Box 6.2.1; for reviews see Jones & Heigenhauser,³ Fitts⁴). The content of this chapter is primarily directed to moderate- to high-intensity exercise because exercise at these intensities produces a significant acid–base disturbance, while exercise at low intensities does not (unless markedly prolonged with underlying dehydration and metabolic abnormalities). Hultman and Sahlin’s 1980 paper still provides the best detailed review of skeletal muscle acid–base balance during exercise.⁵ Previous treatments of

Box 6.2.1 Effects of increased intramuscular $[\text{H}^+]$ and functional consequence(s) in muscle

- Decreased glycogenolytic (phosphorylase) activity^{33,169} → decreased anaerobic adenosine triphosphate (ATP) production → ATP supply is limited → fatigue
- Decreased glycolytic (phosphofructokinase) activity^{33,170,171} → decreased anaerobic ATP production → ATP supply is limited → fatigue
- Decreased pyruvate dehydrogenase activity³³ → decreased aerobic ATP production → ATP supply is limited → fatigue
- Decreased sarcolemmal and sarcoplasmic reticulum Ca^{2+} -ATPase activity^{59,172,173} → elevated cytosolic $[\text{Ca}^{2+}]$ → decreased myosin ATPase activity⁵⁹ → decreased rate of actin–myosin cross-bridge cycling → slowed rate of muscular contraction → fatigue
- Increased $[\text{H}^+]$ increases the [diprotonated inorganic phosphate] → inhibition of actin–myosin cross-bridge interaction resulted^{36,174,175}
- Inhibition of Ca^{2+} binding to troponin C,^{59,176,177} resulting in decreased number of actin–myosin cross-bridges formed → decreased strength of force production → fatigue
- Increased acetyl-CoA, indicative of increased intramuscular tri-acylglycerol hydrolysis³³ → increased citric acid cycle dehydrogenase activities → stimulate aerobic metabolism
- Decreased lactate efflux from muscle cells¹⁷⁸ → prolongation of intracellular acidification by retaining a strong acid anion

muscle acid–base balance emphasizing a physicochemical approach include Lindinger⁶, and Lindinger & Heigenhauser.^{7,8} Thorough reviews of plasma acid–base status have been provided by Constable,⁹ Kowalchuk & Scheuermann,¹⁰ Lindinger et al.,^{11,12} and Johnson et al.¹³ Clinical primers on assessing and treating acid–base disturbances are provided by Whitehair et al.,¹⁴ Constable,¹⁵ Carlson,¹⁶ and Corley & Marr.¹⁷ Hyypä & Pösö¹⁸ and Kingston & Bayly¹⁹ provide brief reviews of the effects of exercise on acid–base status in horses.

In traditional terms, many of us remember being taught that acid–base balance is represented by the relationships among PCO_2 , pH, and the HCO_3^- in blood plasma.^{20,21} While this is true, using only these three variables provides for only a very limited understanding of the factors that contribute to acid–base imbalances. The approach taken within this chapter is to use a comprehensive physicochemical approach to identify the causes or origins of acid–base disturbances during exercise, and to discuss how the disturbance is resolved during recovery from exercise.

While the traditional variables of acid–base — PCO_2 , pH, and the HCO_3^- — are useful in identifying whether an acid–base disturbance is metabolic or respiratory in nature,^{20–22} they are insufficient to identify the physicochemical origins of the acid–base disturbance. It is none the less important for the student of acid–base physiology to be familiar with the concepts presented using the traditional approach, and to be able to use these concepts as an important foundation on which to apply the physicochemical approach. This chapter will emphasize the use of the physicochemical approach as this method provides for a detailed physiological and clinical assessment of acid–base disturbances. It is worth pointing out that the traditional approaches to assessing acid–base status are not incorrect, but rather were a simplification introduced in the 1960s to make use of readily available and relatively simple measurements of PCO_2 and pH. Technological developments from the 1970s have simplified the measurements of the other important acid–base variables in blood plasma and skeletal muscle, allowing us to take a more comprehensive approach.

The physicochemical approach presented here was detailed by Peter Stewart,^{23,24} bears many similarities to earlier work by Peters & Van Slyke,²⁵ and builds on the work of many others, including Hastings, Dill, Lawrence, Henderson, and Siggaard-Andersen. This approach is based on the defined physicochemical properties of electrolyte solutions as detailed in textbooks of physical chemistry.²⁶ Helpful books and

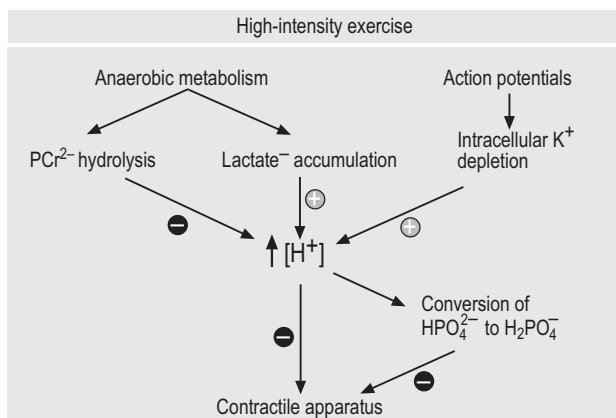
reviews include those by Stewart,^{23,24} Kowalchuk & Scheuermann,¹⁰ Lindinger,⁶ Heigenhauser,²⁷ Jones and Heigenhauser,²⁸ and Constable,¹⁵ as well as software developed by Watson.²⁹

Acidosis and skeletal muscle fatigue

There is no question that high-intensity muscle contraction results in intracellular acidification^{2,30} that generates an extracellular systemic acidosis in the whole organism that can be very pronounced and long-lasting.¹¹ It is also clear that intracellular acidosis and fatigue are associative during high-intensity exercise, with mounting evidence that increased $[H^+]$ reduces the calcium sensitivity of the contractile proteins.³⁰ Furthermore, acidosis imposed prior to the period of high-intensity exercise results in an earlier onset and more pronounced skeletal muscle fatigue.^{31,32} Intracellular acidosis may, however, only exert these effects during high-intensity muscle contraction and recent evidence has shown that the contributions of intracellular acidosis to fatigue process have yet to be fully understood.^{30,33–35} Indeed, Westerblad and colleagues³⁶ have suggested that increased intracellular concentrations of inorganic phosphate may be a more important contributor to muscle fatigue than the increase in $[H^+]$.

Skeletal muscle fatigue is also associated with an increased interstitial $[K^+]$ as a result of rapid rates of K^+ loss through sarcolemmal K^+ channels during the recovery phase of action potentials.³⁷ This increase in interstitial $[K^+]$ results in a marked depolarization of the sarcolemma and decreased contractile force.^{38,39} In contrast to the dogma that we have long been taught, Nielsen et al.³⁴ demonstrated that the loss in both sarcolemmal excitability and tetanic force resulting from elevated interstitial $[K^+]$ (8–12 mEq/L) was actually *reversed* when intracellular acidosis (either 20 mmol/L lactic acid or 50% CO_2) was imposed.³⁴ While these muscles were only stimulated to perform one contraction every 10 min, this allowed a separation between the fatigue associated with repetitive contraction versus that associated with sarcolemmal depolarization and intracellular acidification.

As summarized by Fitts⁴ and Chin & Allen,³⁰ increased $[H^+]$ does contribute to decreased force production during high-intensity muscle contraction (Fig. 6.2.1), and there is reasonably good evidence that these effects occur at the level of

**Fig. 6.2.1**

Overview of events contributing to the intracellular acidosis and fatigue of skeletal muscle during high-intensity exercise. – indicates a decrease in accumulation of function, while + indicates a positive contribution to the increase in $[H^+]$.

- impaired Ca^{2+} binding to troponin C, which therefore impairs the ability of actin to form cross-bridges with myosin
- slowing sarcoplasmic reticulum (SR) Ca-ATPase activity
- increasing the leak of Ca^{2+} from the SR
- a key site of biochemical control within glycogenolysis (decreased glycogen phosphorylase activity) and glycolysis (decreased phosphofructokinase activity).³³

The latter study also demonstrated an increased reliance on fat metabolism to meet the energy demands of contracting muscle during exercise in humans made acidotic by ingestion of 0.3 g/kg ammonium chloride.

It may be concluded that intracellular acidosis may have two main effects that, when taken together, are of long-term benefit for muscle function and survival (prevention of destruction resulting from overuse). First, acidification restores the sarcolemmal excitability and contractility resulting from elevated interstitial $[K^+]$, and the former is very important for cells maintaining the composition of their intracellular environment within physiological limits. Second, the muscle retains the ability to contract with diminished force and rates of contraction and glycogenolysis/glycolysis are slowed as a result of the acidosis. This in turn slows the demand for energy and the production of acid equivalents, while allowing the animal to continue to move if need be.

Assessment of acid–base balance and factors that affect acid–base regulation

The only method capable of fully assessing acid–base balance is the physicochemical approach; therefore it is this system that is detailed below and used within this chapter. Physicochemical characteristics refer to those properties and reactions that are physical and chemical in nature; they proceed in the absence of enzymes and life and occur as a result of the physical and chemical properties of the solvent and solute molecules. Also, biochemical reactions, those catalyzed by enzymes, may alter the physicochemical properties of a solution. However, for the purposes of discussing acid–base balance, biochemical reactions may be considered distinct from physicochemical reactions. The main physicochemical reactions are detailed below.

The advantages and disadvantages of the physicochemical approach are listed in Box 6.2.2. The development and widespread use of ion-selective electrodes and combination blood gas–electrolyte analyzers has greatly simplified the process of obtaining the necessary measurements with the accuracy needed to perform detailed assessments of acid–base balance.^{9,11,40–43} The advantages of this approach lie in the ability to determine quantitatively the physical and chemical origins of acid–base disturbances. This is therefore a very powerful approach and an important step towards understanding acid–base physiology and pathophysiology. This approach provides an essential foundation for the effective treatment of pathological acid–base disorders.

Physicochemical determinants of acid–base balance

Prior to detailing the physicochemical reactions that increase $[H^+]$ within contracting skeletal muscle, it is necessary to provide an introduction to the physicochemical system of acid–base balance. This approach is founded on three underlying physical premises:

1. A dissociated proton molecule (H^+) is only in physical existence for a fleeting instant of time, approximately 10^{-6} s. The proton is highly reactive, associating briefly with negative charges on proteins, OH^- molecules, HCO_3^- molecules, and amino acids, to name a few. The proton is therefore very

Box 6.2.2 Disadvantages and advantages of the physicochemical approach to determination of acid–base balance

Disadvantages

- Requires accurate measurement of many variables including plasma PCO_2 , $[Na^+]$, $[K^+]$, $[Cl^-]$, $[lactate^-]$, $[plasma\ protein]$, and, in muscle, additionally $[A_{tot}]^{40}$
- Requires advanced calculator or computer to perform calculations
- Requires consideration of physical chemistry, biochemistry, and physiology — it is truly an integrative approach

Advantages

- Provides for detailed analysis of why changes in $[H^+]$ and $[HCO_3^-]$ occurred, giving insight into the pathophysiology of any type of acid–base disorder
- Allows for the determination of the individual independent variables, i.e. $[Na^+]$, $[lactate^-]$, $[plasma\ protein]$. . . that is, the origin of the acid–base disturbance
- Identification of the origins of the acid–base disturbance allows one to determine the physiological or biochemical mechanism(s) responsible for the alteration(s) in independent variables
- Knowledge of the physicochemical origins and physiological/biochemical mechanism(s) behind the acid–base disturbance allows for the development and administration of effective treatment strategies for correcting the acid–base disturbance with minimal untoward side-effects

unlike inorganic electrolytes such as Na^+ , K^+ , and Cl^- , which are relatively unreactive.

2. Protons are a main constituent of water, the most prevalent molecule within the body. Water thus provides an almost limitless source of H^+ for biochemical and physicochemical reactions. Protons are part of the solvent that comprises the milieu of the body. It is because of the ability of water to so rapidly dissociate and reassociate H^+ and OH^- that it is the ‘universal’ solute.
3. Because of these physical attributes of protons and water, it is physically impossible to add protons to a physiological solution without adding water. Take hydrochloric acid (HCl) as an example. HCl exists in aqueous form and is characterized by very high concentrations of Cl^- and H^+ in solution. The H^+ is an integral part of the aqueous system. As described below, it is the strong acid anion Cl^- that

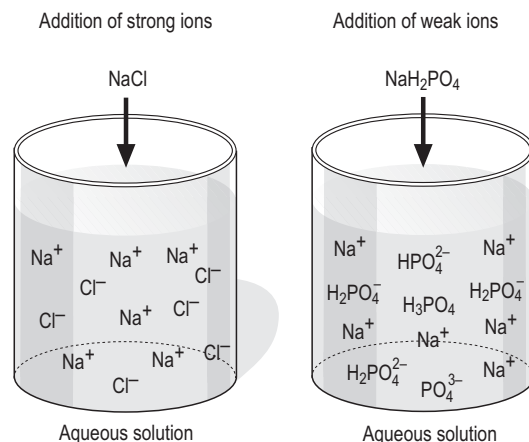


Fig. 6.2.2

Representation of strong ions and weak ions in an aqueous solution. The addition of NaCl to an aqueous solution results in the complete dissociation into the strong ions Na^+ and Cl^- . In contrast, the addition of sodium phosphate results in the complete dissociation of all of the sodium into Na^+ , but the phosphate is capable of reacting with H^+ in solution to form the following weak ions: H_3PO_4 , $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} .

makes this solution so acidic. The strong acid anion Cl^- can be neutralized by the addition of an equivalent amount of the strong base cation Na^+ to the solution, but without an accompanying acid anion such as Cl^- , HCO_3^- , or $H_2PO_4^-$. Thus NaOH would be added; the strong anions Cl^- and Na^+ remain fully dissociated in solution while there occurs a rapid reaction between H^+ and OH^- that decreases $[H^+]$. The resultant solution is saline at neutral pH.

The physicochemical approach to acid–base balance recognizes that three groups of independent variables determine the concentrations of the traditional acid–base variables pH and $[HCO_3^-]$:

- the strong ion difference ([SID]), which represents the sum (charge considered) of the concentrations of strong acid anions and strong base cations
- the total weak acid concentration (A_{tot}), which represents the sum (charge considered) of the weak acids and bases
- the carbon dioxide (CO_2) concentration, which is usually measured and used as the partial pressure of CO_2 (PCO_2) (Fig. 6.2.2).

Strong ions and strong ion difference

The terms ‘strong acid anion’ and ‘strong base cation’ were introduced in the preceding section and they will

be defined here. The term ‘strong’ refers to the fact that the ion will be fully dissociated, or nearly so, in aqueous solutions (Fig. 6.2.2). Most of the inorganic ions are ‘strong’ and hence nearly fully dissociated within the body fluids (Box 6.2.3). Some organic ions are also strong, such as lactate[−] (acid dissociation constant of 3.9) and phosphocreatine^{2−} (PCr^{2−}, acid dissociation constant of 4.5). Anions possess negative charge whereas cations possess positive charge. An anion is an acid by definition because the addition of that strong anion, in the absence of an accompanying strong base, will result in acidification of the solution. Using the example of HCl above, the addition of HCl to plasma will result in acidification. Similarly, the addition of lactate will also result in acidification. In contrast, the addition of the strong base Na⁺ in the absence of accompanying strong anion (as NaHCO₃) will result in alkalization. The values for the key variables used in the physicochemical assessment of acid–base balance, for resting horses, are provided in Table 6.2.1.

The concentrations of strong acid anions and strong base cations within a fluid compartment are summed, with consideration of the charge, to yield the strong ion difference [SID].

Within plasma and the extracellular fluid compartment the [SID] can be calculated as:

$$[\text{SID}] \text{ (mEq/L)} = ([\text{Na}^+] + [\text{K}^+] + [\text{Mg}^{2+}] + [\text{Ca}^{2+}]) - ([\text{Cl}^-] + [\text{lactate}^-] + [\text{SO}_4^{2-}])$$

Note that it is the free or ionized concentrations of the divalent ions that must be used, and not the total concentration; considerable amounts of the divalent ions are bound to plasma proteins or to each other. The concentrations measured using ion-selective electrodes are those of the free or ionized or dissociated ion in the aqueous portion of the solution (i.e. mEq/L of plasma water), so long as the instrument does not use a calculation to modify the ‘concentration’ to total (not free) concentration in units of mEq/L of plasma. Thus, while these divalent ions are ‘strong’, the interactions with charged moieties on protein molecules remove some of the ion from solution. In practice, the free concentrations of the divalent cations and anions are approximately equivalent and can be ignored, leaving:

$$[\text{SID}]_{\text{plasma}} \text{ (mEq/L)} = ([\text{Na}^+] + [\text{K}^+]) - ([\text{Cl}^-] + [\text{lactate}^-])$$

In some treatments of acid–base balance using the physicochemical approach, [lactate[−]] is also ignored. However, [lactate[−]] cannot be ignored in the exercising and recovering animal.

Box 6.2.3 A summary of acid–base terminology

The following definitions are placed in order of functional similarities, as opposed to alphabetical order:

- **Base:** any cation in biological fluids²²
- **Buffer base:** base equivalent to the sum of buffer anion concentrations (including [HCO₃[−]]) in mEq/L²²
- **Base excess/deficit:** represents the accumulation of non-volatile base/acid in the blood (excludes plasma [HCO₃[−]] and blood hemoglobin concentration)^{179,180}
- **Alkali (alkaline) reserve:** the proton-buffering ability of plasma bicarbonate when bases or non-volatile acids are added to or taken from the body fluids²²
- **[A_{tot}]:** a physicochemical term that defines the total concentration of weak anions in solution²³
- **[SID]:** a physicochemical assessment term that refers to the sum of all strong base cations minus the sum of all strong acid anions:²³

$$[\text{SID}] = \Sigma[\text{strong base cations}] - \Sigma[\text{strong acid anions}]$$

- **Strong ions:** those ions that are fully dissociated, or nearly so, in physiological solutions. In general, if the dissociation constant is ≤4.5, then the molecule is considered to be a strong anion; if the dissociation constant is >9, then the molecule is considered to be a strong cation
- **Anion gap:** a traditional term that is defined as:
Anion gap = ([Na⁺] + [K⁺]) − ([Cl[−]] + [HCO₃[−]])
- **Strong ion gap:** a term coined by Constable et al¹⁸¹ as an alternative way of determining the concentration of unmeasured strong ions in plasma:
Strong ion gap = 2.24 × total [protein] (g/dl) / (1 + 10^(6.65−pH)) − AG, where AG is the anion gap
- **Unmeasured anions:** unmeasured anions contribute to the anion gap, strong ion difference, and strong ion gap. The unmeasured anions include both strong (SO₄^{2−}, some amino acids, pyruvate) and weak (inorganic phosphate, carbonate, carbamates, some amino acids) anions. The negative charges on plasma protein contribute to the anion gap and strong ion gap, but this is usually a ‘measured’ anion

Table 6.2.1 Physiologically important acid–base variables, and their concentrations, in arterial plasma and skeletal muscle of horses at rest

	Plasma		Skeletal muscle	
	'Normal' value	Normal range	'Normal' value	Normal range
Dependent variables				
[H ⁺] nEq/L	40	33–45	100	71–126
pH	7.40	7.35–7.48	7.0	6.90–7.15
[HCO ₃ ⁻] mEq/L	28	22–34	10	8–12
Independent variables				
PCO ₂ mmHg	40	35–45		
[total CO ₂] mmol/L	30	23–36	10 ^a	8–12 ^a
Strong ions				
[SID] mEq/L	40	37–43	110 ^c	60–130 ^c
[Na ⁺] mEq/L	140	132–146	15	
[K ⁺] mEq/L	3.7	2.7–4.7	140	
[Ca ²⁺] mEq/L	2.5	2–3	0.4	
[Mg ²⁺] mEq/L	1.0	0.5–2.0	5	
[Cl ⁻] mEq/L	105	99–109	10	
[lactate ⁻] mEq/L	1.0	0.5–1.5	1.5	1.0–2.0
[PCr ²⁻] mEq/L	na	na	15	12–18
[SO ₄ ²⁻] mEq/L	0.5	0.3–0.7	–	–
Weak ions				
[A _{tot}] mEq/L	12	11–13	140	120–170
[plasma protein] g/dL	5.5	5.0–6.0	na	na
[albumin] g/dL			na	na
[globulins] g/dL			na	na
[HPO ₄ ²⁻] + [H ₂ PO ₄ ⁻] mmol/L	2.7	2.0–3.5	8 ^b	7–9 ^b
Carnosine mmol/L	na	na	6 ^b	3–9
Protein histidine concentration mmol/L	–	–	46 ^b	30–60

^aSahlin et al¹⁸² — human muscle.^bHultman & Sahlin⁵ — human muscle.^cLindinger.⁶

na = not applicable.

Within skeletal muscle, PCr²⁻ and Mg²⁺ must be used within the equation because their free concentrations are large and change substantially during exercise:

$$[\text{SID}]_{\text{muscle}} (\text{mEq/L}) = ([\text{Na}^+] + [\text{K}^+] + [\text{Mg}^{2+}]) - ([\text{Cl}^-] + [\text{lactate}^-] + [\text{PCr}^{2-}])$$

The strong ions are important determinants of the concentrations of [H⁺] and [HCO₃⁻] because they directly affect the associated state of H₂O, and thereby determine the concentrations of H⁺ and ⁻OH.

A decrease in the [SID] (without concurrent change in PCO₂ or [A_{tot}]), due to either a decrease in strong cation concentration or an increase in strong anion concentration, will increase [H⁺] and decrease [HCO₃⁻] — an acidification occurs. Conversely, an increase in [SID] has an alkalinizing effect and decreases [H⁺] and increases [HCO₃⁻].

Weak acids and bases, and [A_{tot}]

The term 'weak' refers to those anion acids and cation bases that are not fully dissociated in solution. Thus when sodium phosphate (Na₂HPO₄) is added to an aqueous solution, two Na⁺ are added, fully dissociate and a weak anion HPO₄²⁻ is added. In contrast to Na⁺, the HPO₄²⁻ cannot achieve full dissociation due to reactions of the molecule with H⁺ within the solution. Thus the HPO₄²⁻ is also partially and instantaneously transformed into H₃PO₄, H₂PO₄⁻, and PO₄³⁻ (see Fig. 6.2.2). This physical attribute of phosphate is what makes phosphates, and many other weak acid anions such as bicarbonate and albumin, good proton 'buffers'. The predominant weak acid anion in plasma and extracellular fluid (ECF) is albumin, while the predominant weak acid anions within skeletal muscle cells are the histidine residues on proteins.

Table 6.2.2 Values of the constants used within the acid–base equations

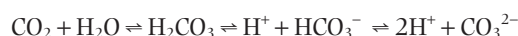
Parameter	Constant	Reference
K_A — plasma	2.11 or 2.22×10^{-7} Eq/L	9,43
K_A — resting muscle	1.64×10^{-7} Eq/L	7,8
K_A — exercised muscle	1.98×10^{-7} Eq/L	7,8
K_3	6.0×10^{-11} Eq/L	23
K_C	2.46×10^{-11} (Eq/L) ² /mmHg	23
K'_w	4.4×10^{-14} (Eq/L) ²	23

The main weak acids and bases within the ECF compartment are albumin, globulin, phosphate, and bicarbonate. Bicarbonate, however, is part of the CO_2 system and thus is not used in the calculation, or estimation, of $[\text{A}_{\text{tot}}]$. As with the strong ions, the weak ions also directly affect the concentrations of H^+ and HCO_3^- in solution. Within skeletal muscle it is primarily the histidine moieties on proteins that contribute to $[\text{A}_{\text{tot}}]$, with creatine, Pi, ATP, and other molecules also contributing.⁶ While it is theoretically possible to measure the concentrations of weak acids and bases in both extracellular and intracellular fluid compartments, this tends to be prohibitive and appears not to be necessary to be able to estimate acid–base state effectively. Rather, an effective $[\text{A}_{\text{tot}}]$ and apparent dissociation constant K'_a have been determined in equine plasma and rat skeletal muscle (Table 6.2.2). A value for $[\text{A}_{\text{tot}}]$ has not been determined in equine or human skeletal muscle. Muscle $[\text{A}_{\text{tot}}]$ is equivalent to the non-bicarbonate proton-buffering capacity of adult rat plantaris muscle,^{7,8} and is similar to that of human vastus lateralis.⁵ When rat plantaris values for $[\text{A}_{\text{tot}}]$ and K'_a were applied to human muscle, reasonable data were generated.⁶ Equine muscle, compared to human muscle, has a much greater non-bicarbonate proton-buffering capacity: 43 mEq/kg dry muscle⁻¹.pH⁻¹ in trained humans, versus 58 and 93 mEq/kg⁻¹.pH⁻¹ in untrained and trained equine skeletal muscle, respectively.⁴⁴ Assuming proportionality with rat hindlimb skeletal muscle (buffer capacity of ~40 mEq/kg.pH⁻¹ = $[\text{A}_{\text{tot}}]$ of ~140 mmol/L,⁶ this translates to an $[\text{A}_{\text{tot}}]$ of ~315 mmol/L in trained equine muscle.

The carbon dioxide system

The concentration of CO_2 is the third independent variable of acid–base balance. Carbon dioxide is effectively a strong acid, and because it is a major end-product of cellular respiration, is often referred to as a respiratory acid. Also, the primary means for eliminating excess CO_2 from the body is through the respiratory system.^{13,28}

Carbon dioxide is a strong acid by virtue of its ability to combine with water to increase the concentration of H^+ while at the same time increasing the weak acid $[\text{HCO}_3^-]$. This reaction effectively acidifies the solution to which CO_2 has been added. The majority (about 95%) of the total CO_2 within the body is in the form of HCO_3^- , with much smaller amounts of H_2CO_3 , CO_3^{2-} , dissolved CO_2 ($\text{CO}_{2(\text{d})}$), and some that is bound to amino groups on protein to form carbamino compounds. The chemical reactions involved in the hydration and dehydration of CO_2 are:



Solving equations to determine acid–base balance

With this background, the following five mass action equations and one equation expressing electrical neutrality of solutions describe the physicochemical characteristics of any aqueous, physiological solution:^{23,24}

Water dissociation:

$$K'_w = [\text{H}^+] \cdot [\text{OH}^-]$$

Weak electrolyte system:

$$K_A \cdot [\text{HA}] = [\text{H}^+] \cdot [\text{A}^-]$$

$$[\text{A}'_{\text{tot}}] = [\text{HA}] + [\text{A}^-]$$

Carbon dioxide system:

$$K_C \cdot P_{\text{CO}_2} = [\text{H}^+] \cdot [\text{HCO}_3^-]$$

$$K_3 \cdot [\text{HCO}_3^-] = [\text{H}^+] \cdot [\text{CO}_3^{2-}]$$

Electrical neutrality:

$$[\text{SID}] + [\text{H}^+] - [\text{HCO}_3^-] - [\text{A}^-] - [\text{CO}_3^{2-}] - [\text{OH}^-] = 0$$

It is noteworthy that $[\text{H}^+]$ appears in each of these equations and its dependence on the concentrations of strong and weak acids/base and CO_2 is evident. These six equations can be combined into a single equation that may then be solved for $[\text{H}^+]$ when the

three independent variables and the constants are known:^{23,24}

$$[H^+]^4 \{ \{K_A = [SID]\} [H^+]^3 - \{K_A ([SID] - [A_{tot}]) - (K_C P_{CO_2} + K'_W)\} [H^+]^2 - \{K_A (K_C P_{CO_2} + K'_W) + K_3 K_C P_{CO_2}\} [H^+] - K_A K_3 K_C P_{CO_2} = 0$$

Contracting skeletal muscle: proton-generating and removing reactions

When considering the acid–base changes that occur in blood during exercise, it is important to have an understanding of the changes that occur within skeletal muscle because that tissue forms 40–60% of the mass of the horse.⁴⁵ Contracting skeletal muscle generates the disturbance^{11,41} and non-contracting cells are capable of ameliorating the disturbance.^{42,46} The role of non-contracting muscle may be small to negligible in horses performing moderate- to high-intensity exercise because most skeletal muscles are used for locomotion and maintenance of posture. That is in contrast to bipedal humans, where many activities require leg muscles and leave many other muscles relatively inactive. This section will thus focus on the time course and magnitude of changes that occur within contracting skeletal muscle, primarily gluteus medius, during moderate- to high-intensity exercise.

Exercise is a consequence of muscular contraction, and muscular contraction results in an increase in cellular energy demand compared to the resting state. The increased energy demand is due to activation of myosin ATPase needed for release of actin–myosin cross-bridge interaction, increased activity of SR Ca^{2+} -ATPase resulting from increased cytosolic $[Ca^{2+}]$, and increased rates of Na,K-ATPase activity needed to maintain transmembrane Na^+ and K^+ gradients and repolarization of the muscle membrane potential.

The acid–base changes that occur within contracting skeletal muscle and in blood during exercise are the results of the biochemical (metabolic) and physicochemical reactions that occur within contracting muscle cells. The onset of muscular contraction sets into motion a series of biochemical events that result in stimulation and inhibition of numerous metabolic pathways. Those pathways within the aerobic energy systems are relatively slow to increase, whereas those

of the anaerobic pathways (ATP utilization, phosphocreatine degradation, glycolysis) increase rapidly.⁴⁷ Thus the onset of exercise (rest to work transition) may be associated with muscular acidification for reasons described below. Similarly, transitions from low to high work rates, as well as exercise at moderate to high intensities, result in increased rates of anaerobic metabolism. Full activation of aerobic pathways may be achieved within minutes of the onset of exercise, but until this is achieved anaerobic pathways continue to supply ATP. Activation of aerobic metabolism results in increased mitochondrial respiration with CO_2 production; while this CO_2 is acidic, its rate of production and removal from the cell can easily be matched by CO_2 elimination rates at the lung.^{13,28} Therefore aerobic CO_2 production can be ignored in most discussions of the acid–base changes of exercise.

Muscle characteristics and acid–base

Skeletal muscle is composed of different fiber types, some of which produce acid equivalents at high rates (the anaerobic, fast-twitch, glycolytic fibers) and others that do not (the aerobic, slow-twitch, oxidative fibers). Fiber types continue to be classified on the basis of their twitch characteristics, on their oxidative/glycolytic capacities, and on their myosin heavy chain composition.⁴⁸ The acid–base changes that occur reflect the fiber type composition of the contracting muscles, and thus reflect breed differences and type of activity performed.

Within individual muscle groups, such as the well-studied gluteus medius of equids, skeletal muscle fibers of different composition are in close proximity and form integrated functional units that are selectively recruited by appropriate motor units depending on the locomotory requirements of the animal. Muscle fibers with high oxidative capacity, that have low glycolytic capacity and slow contractile properties with low myosin ATPase activity, are fatigue-resistant and primarily function in the maintenance of posture (Table 6.2.3). These slow-twitch oxidative fibers have the ability to oxidize all the pyruvate generated from glycolysis and, during exercise, they have the ability to take up and oxidize lactate released into the interstitium from nearby glycolytic muscle fibers — the intramuscular lactate shuttle.⁴⁹ At the other extreme, fibers of high glycolytic capacity with low oxidative capacity have fast contractile properties with high myosin

Table 6.2.3 Skeletal muscle fiber types in horses and relationship to acid–base balance

	Fiber type		
	SO — type I	FOG — type IIA	FG — type IIB
% of total fibers ^{a,b}	15	45	40
Total H ⁺ buffer capacity ^a	88	98	130
Carnosine H ⁺ buffer capacity ^a	18	58	60
Carnosine % of total ^a	20	29	45
Carnosine content ^a	54 ± 15	85 ± 15	180 ± 15
Glycogen phosphorylase activity ^b	122 ± 20	71 ± 20	172 ± 20
Citrate synthase activity ^b	168 ± 15	167 ± 15	37 ± 15
Glycogen content ^c	+	++	++

SO, slow oxidative; FOG, fast-twitch oxidative glycolytic; FG, fast-twitch glycolytic.

^{a,b}Averaged data from Sewell et al.⁵⁷ ($n = 20$ 2–3-year-old racing Thoroughbreds); Sewell et al.⁵⁰ ($n = 50$ 2–3-year-old racing Thoroughbreds).

^aData from Sewell et al.⁵⁷

^bData from Sewell et al.⁵⁰

^cData from Quiroz-Rothe and Rivero.¹⁸³

ATPase activity. These fibers function to generate power at high rates for high-intensity sprinting, jumping, and pulling activities. The majority of the acid–base disturbance that occurs during high-intensity exercise is generated within the fast-twitch glycolytic (type IIB or type IID/X) that are endowed with a high glycogen content, high activities of glycogen phosphorylase and lactate dehydrogenase, and a high content of carnosine to buffer metabolically generated H⁺. These fibers also fatigue rapidly due to the loss of membrane excitability, as well as their high rate of intracellular acidification⁸ that effectively downregulate muscle fiber function at multiple membrane and intracellular sites.⁴

It is noteworthy that Quarter Horses and Thoroughbreds, the two fastest horse breeds, have the lowest proportion of slow oxidative (type I) fibers and high proportions of both fast-twitch oxidative glycolytic (type IIA) and fast-twitch glycolytic (type IIB, also known as type IIX) fibers. These fast-twitch fibers are also known to have high activities for glycogen phosphorylase,⁵⁰ catalyzing the initial reaction in glycogenolysis, and lactate dehydrogenase^{51,52} for converting pyruvate to lactate. Indeed, in racing Thoroughbreds and Standardbreds there is a high degree of correlation between type IIB fiber proportion and lactate accumulation:⁵¹ fiber type-specific lactate content 6 min after the end of 1200–2700 m races was greater in gluteus medius type IIB fibers (97.3 ± 2.1) than in type I (82.6 ± 3.8 mmol/kg dry muscle; $n = 8$), with type IIA fibers intermediate (93.6 ± 2.1). This is con-

sistent with observations that type IIB fiber proportion is positively correlated with elevated plasma [lactate[−]] during submaximal and maximal exercise in Standardbred trotters.^{52–54} Of interest and importance to muscle acid–base regulation is the observation that in type I fibers [lactate[−]] there was only 15 mEq/kg dry muscle (equivalent to about 3 mEq/L) less than in type IIB fibers. This supports the idea that lactate produced in type IIB fibers diffuses out of these fibers into the interstitium where the lactate can be taken up by oxidative fibers^{49,54,55} to be used as a fuel source both during and following exercise.

The fast-twitch fibers are also endowed with an important physicochemical feature that aids in the regulation of intracellular acid–base balance: there is an increasing content of the histidine dipeptide, carnosine, with increasing glycolytic capacity. Carnosine has a pK_a ~6.9 and, as such, is an effective H⁺ buffer and contributes substantively to the non-bicarbonate H⁺ buffer capacity of muscle, particularly in fast-twitch glycolytic fibers.⁵⁶ Indeed, if the contribution of carnosine to non-bicarbonate buffering is removed, then each of the different fiber types has similar non-bicarbonate H⁺ buffering capacities; thus carnosine confers all of the fiber type difference in non-bicarbonate H⁺ buffering capacity.^{50,57} Quarter Horses have a higher carnosine content (39.2 ± 1.8 mmol/kg wet muscle; $n = 6$) than Thoroughbreds (31.3 ± 2.9 mmol/kg wet muscle; $n = 6$) and Standardbreds (27.6 mmol/kg wet muscle; $n = 5$).⁵⁸ The high H⁺ buffering capacity of glycolytic fibers is important given the high rates of

metabolic H^+ production resulting from ATP hydrolysis, glycogenolysis, and glycolysis (described above). Without the ability to buffer this rapidly produced H^+ during exercise, cellular $[H^+]$ would rapidly increase to concentrations inhibitory for actin–myosin cross-bridge cycling^{4,59} and for many metabolic reactions.^{4,60,61} Buffering of H^+ within the cells in which it is produced also reduces the extent of extracellular acidification that occurs. Cessation of activity and resynthesis of ATP and glycogen results in a lowering of intracellular $[H^+]$ and a decrease in the quantity of H^+ buffered by histidine groups within the cell.

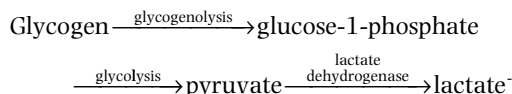
Biochemical origins of H^+ changes: anaerobic metabolism and muscle acidification/alkalinization

Muscular acidification occurs as a necessary consequence of providing ATP at high rates, using anaerobic metabolic pathways.^{62,63} Rapid increases in anaerobic metabolism are needed during rest-to-work transitions and during transitions from work of lower intensity to higher-intensity work, such as occurs during cutting and jumping. The rate-limiting enzymes of anaerobic metabolic pathways are rapidly activated compared to the duration required for peak activation of key rate-limiting enzymes of the aerobic metabolic pathways.

ATP hydrolysis by myosin ATPase, Ca^{2+} -ATPase, and the Na^+,K^+ -ATPase results in the net production of H^+ .⁶²



The anaerobic reactions of glycogenolysis and glycolysis are also rapidly activated, resulting in the production of 3 ATP for each lactate⁻ produced. Lactate⁻ is produced because of an accumulation of pyruvate at the end of the glycolytic pathway, resulting in conversion of pyruvate to lactate by the enzyme lactate dehydrogenase:

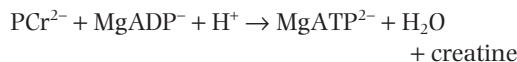


For each mole of lactate⁻ produced from glycolysis, 0.36 moles of H^+ are produced, while for each mole of lactate⁻ produced from glucose-6-phosphate (arising from glucose transport into the cell, with the production of 2 ATP), 0.575 mole of H^+ is produced.⁶²

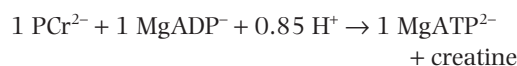
The ATP produced from these anaerobic reactions is rapidly hydrolyzed, resulting in the production of

0.425 H^+ for each ATP hydrolyzed (see above). This reaction, combined with those that produce lactate⁻, results in a nearly 1:1 stoichiometry for lactate⁻: H^+ .⁶²

When ATP utilization rates are high, phosphocreatine (PCr^{2-}) is hydrolyzed, resulting in the consumption of H^+ .⁶²



The rates of hydrolysis of ATP and PCr^{2-} are closely coupled and occur simultaneously as long as there is PCr^{2-} to hydrolyze. In simplified form, the combined ATP hydrolysis reaction and the PCr^{2-} hydrolysis reaction is:⁶²

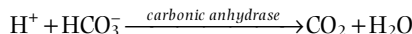


Thus, for each mole of PCr^{2-} hydrolyzed to regenerate a mole of $MgATP^{2-}$, 0.85 mole of H^+ is generated.

Examination of this sequence of biochemical reactions shows that the H^+ produced by ATP hydrolysis is more than counteracted by the H^+ consumed by PCr^{2-} hydrolysis, resulting in the well-known decrease in intracellular $[H^+]$ at the onset of exercise. Within the first few minutes of exercise there occurs a decreased reliance on PCr^{2-} hydrolysis to regenerate ATP, as ATP is increasingly produced from glycogenolysis/glycolysis as well as from aerobic sources. When exercise is continued above the lactate threshold, the combined reaction of ATP hydrolysis and lactate⁻ production produces nearly equivalent amounts of lactate⁻ and H^+ within skeletal muscle.

Most of the H^+ produced does not remain free in solution but binds to negatively charged sites, primarily histidine residues, on weak acids and bases such as intracellular proteins and is thus buffered.⁵ Therefore, despite micromolar and millimolar changes in the concentrations of intracellular metabolites and strong ions, proton buffering by intracellular proteins limits $[H^+]$ changes to the nanomolar range.

In addition to reacting with intracellular proteins, H^+ also readily reacts with HCO_3^- (bicarbonate buffer system):



When intracellular $[H^+]$ increases as a result of increased anaerobic metabolism, this reversible reaction, catalyzed by carbonic anhydrases, produces CO_2 and water. The CO_2 itself is acidic and is removed from the cell by carbonic anhydrases and possibly by diffusion. As may be appreciated, during high-intensity exercise, when proton generation rates are very high,

the bicarbonate buffer system is very limited with respect to its ability to remove H^+ from solution.

Because increased intracellular $[H^+]$ contributes to skeletal muscle fatigue, prevention or delayed onset of fatigue can only occur if H^+ is removed. Both the bicarbonate and non-bicarbonate (protein) buffer systems are limited. However, the cell can transport Na^+ , a strong base cation, into the cell (in exchange for H^+) using the Na^+-H^+ exchanger, and possibly augment intracellular HCO_3^- using a $Cl^-HCO_3^-$ exchanger.⁶⁴ In addition to these mechanisms, the outward transport of lactate⁻ also contributes to an alkalinizing effect, as detailed below.

In summary, H^+ is produced from the hydrolysis of ATP and from the production of lactate⁻. In contrast, the hydrolysis of PCr^{2-} at the onset of muscle contraction consumes H^+ to a greater extent than the H^+ produced through ATP hydrolysis, resulting in intracellular alkalization. With continuation of moderate- to high-intensity muscle contraction, H^+ and lactate⁻ continue to be produced, resulting in intracellular acidification. Much of the H^+ is buffered by intracellular proteins or reacts with HCO_3^- to produce CO_2 and H_2O . CO_2 is removed from the cell by diffusion across the plasma membrane and by carbonic anhydrases.

Physicochemical origins of $[H^+]$ changes in skeletal muscle during exercise

In addition to the biochemical reactions summarized above, physicochemical changes within the intracellular compartment of contraction muscle also contribute to the acid–base changes of exercise. There is involvement of each of the three independent physicochemical variables, although the changes in the concentrations of strong ions predominate, as will be exemplified below. The physicochemical descriptions complement the biochemical descriptions provided above.

Muscle $[SID]$ during exercise

The time course and magnitude of changes in muscle $[SID]$, and of the factors contributing to it, have not been well studied in horses. This is due in large part to the fact that muscle biopsies cannot truly be taken during exercise — the exercise must be stopped and the horse restrained to obtain a useful piece of muscle

safely. Also, most studies have taken post-exercise muscle biopsies between 2 and 20 min after cessation of exercise, during which time there are substantial changes in organic and inorganic strong ions. Accordingly, studies that measured muscle $[K^+]$ showed no change⁶⁵ or a small increase⁶⁶ after brief periods of high-intensity exercise and this may be attributed to rapid recovery processes. The limited equine data will be used with what is known from human experiments to profile the changes occurring within muscle during exercise and recovery. The focus will be on high-intensity exercise because the changes are more pronounced and hence somewhat easier to follow. Changes with moderate-intensity exercise are attenuated compared to those occurring with high-intensity exercise.^{54,67,68} Muscle acid–base responses to low-intensity exercise have not been specifically studied and, indeed, changes in metabolites that affect acid–base state are minimal.^{69,70} With prolonged endurance exercise it is expected that the major players would be $[SID]$ changes resulting from net K^+ loss, with Na^+ and Cl^- gain, water loss or gain,⁷¹ and changes in $[A_{tot}]$ primarily resulting from changes in intracellular water content.

The main variables changing within muscle during exercise that affect $[SID]$ are $[PCr^{2-}]$, $[lactate^-]$, $[K^+]$ ⁶ and water content.⁷² The osmotic shift of water from plasma and non-contracting tissues into contracting muscle at the onset of exercise is very large and rapid and can be attributed to the accumulation of osmolytes such as creatine, inorganic phosphate, and lactate⁻.⁷³ This fluid shift produces a large decrease in plasma volume (see below) and the 10% increase in intracellular volume at the end of 2 min of high-intensity exercise in horses⁷² effectively dilutes intracellular metabolites and electrolytes and reduces $[A_{tot}]$. It is the actual concentrations of these variables that determine the acid–base state of the cell at any given point in time.

When working from metabolite or electrolyte data expressed in mmol/kg dry muscle or mmol/kg wet muscle it is necessary to convert to units of mmol/L or mEq/L of intracellular water. The water content of resting, non-exercised skeletal muscle is 0.75 L/kg wet muscle⁷⁴ and increases by about 10% with high-intensity exercise;⁷² resting muscle thus has a wet:dry weight ratio of ~4, which increases to ~4.5 with high-intensity exercise. Therefore, a resting PCr^{2-} content of 85 mmol/kg dry muscle equates to 21 mmol/kg wet muscle (85/4) and 28 mmol/L (21/0.75) or 56 mEq/L. The doubling from 28 mmol/L to 56 mEq/L recognizes the divalent negative charge on PCr^{2-} .

With the onset of exercise, the initial few seconds of contraction result in the rapid hydrolysis of PCr^{2-} to regenerate ATP being used by myosin-, Ca- and Na,K-ATPases. The hydrolysis of PCr^{2-} effectively removes a strong acid anion from solution, which increases intracellular [SID] and has an alkalinizing effect (Fig. 6.2.3). The alkalinizing effect resulting from PCr^{2-} hydrolysis is short-lived. With high-intensity exercise, the high rates of H^+ and lactate $^-$ (a strong acid anion that decreases [SID]) production rapidly acidify the intracellular environment. Concurrent increases in [lactate $^-$]^{41,61,67} and decreases in $[\text{K}^+]$ ⁷⁵ effectively decrease [SID], which accounts for the majority of intracellular acidification during high-intensity exercise.⁸ As may be expected, the accumulation of muscle lactate $^-$ contributes substantially to the exercise-induced acidosis because lactate $^-$ is a strong acid anion that increases $[\text{H}^+]$. Intracellular lactate $^-$ concentrations greater than 60 mEq/L (234 mmol/kg dry weight) have been reported in the gluteus medius of trained Standardbreds after trotting 1600 m at a high speed of 11 m/s.⁷⁶ With lower-intensity exercise, PCr^{2-} is resynthesized during the subsequent period of steady-state exercise, thus contributing to decreases in [SID] and intracellular acidification.

The physicochemical factors contributing to intracellular acidification will now be examined in more detail. Thoroughbreds exercising at high intensity hydrolyzed 32% of PCr^{2-} in 40 s (600 m sprint),⁶¹ and 42% after 5 min at 100% of peak VO_2 .⁶⁸ In these studies, muscle biopsies were obtained within 60 s of stopping exercise and thus the amount of post-exercise PCr^{2-} resynthesis was low at the time of muscle sampling. In resting equine muscle, $[\text{PCr}^{2-}]$ of 21 mmol/kg wet muscle^{61,67,74} equates to 56 mEq/L (21 mmol/kg wet muscle/0.75 L/kg intracellular

water).⁷⁴ Thus, after 40 s of sprinting, muscle $[\text{PCr}^{2-}]$ was reduced by 18 mEq/L, which effectively raises [SID] by 18 mEq/L; by virtue of this increase in [SID] it is evident that PCr^{2-} hydrolysis has an alkalinizing effect. This effect, however, is completely offset by the accumulation of lactate $^-$, with muscle [lactate $^-$] reaching 45 mEq/L.⁶¹ Very similar results for PCr^{2-} degradation were reported by Lindholm & Saltin⁶⁷ in five racing Standardbreds completing 2100 m in 178 s; however, these trained race horses experienced considerably less muscle lactate $^-$ accumulation (~20 mEq/L), a likely training adaptation.

Combining the effects of changes in $[\text{PCr}^{2-}]$ and [lactate $^-$] decreases [SID], which has a net acidifying effect. This effect alone, however, only accounts for about 50% of the observed decrease in muscle intracellular pH⁸ from 7.01 to 6.86;⁶¹ this is equivalent to an increase in $[\text{H}^+]$ from 98 to 138 mEq/L. The other contributor to the decrease in [SID] is the rapid and pronounced decrease in $[\text{K}^+]$ during high-intensity exercise.^{71,75} In the gluteus medius of Standardbreds at rest, intracellular $[\text{K}^+]$ is 122 ± 7 mEq/L (92 ± 5 mEq/kg wet weight, with a water content of 75%).⁷⁴ There are no reports of equine muscle $[\text{K}^+]$ during exercise^{65,66} and, on the basis of the large increases in plasma $[\text{K}^+]$ seen during exercise in horses,⁷⁷ it is expected that horses experience similar rates and magnitudes of muscle K^+ loss as do humans. In humans, 30 s of high-intensity exercise decreased muscle $[\text{K}^+]$ by 20 mEq/L and, with increases in muscle $[\text{Na}^+]$ and $[\text{Cl}^-]$ balancing each other, the decrease in [SID] is thus approximately $20 + 11 = 31$ mEq/L. Based on titrimetric studies conducted on rat fast-twitch skeletal muscle, the 31 mEq/L decrease in [SID] is sufficient to account for about 75% of the increase in $[\text{H}^+]$.⁶ In summary, using this example of muscle after 40 s of high-intensity exercise, the decrease in [SID] can account fully for the increase in $[\text{H}^+]$. Coincidentally, the decrease in [SID] approximates the increase in [lactate $^-$], and the decrease in $[\text{PCr}^{2-}]$ was similar to the decrease in $[\text{K}^+]$. Further study is needed to determine if this relationship holds during high-intensity exercise. If it does, it means that decreases in [SID] during high-intensity exercise may be estimated from the increase in [lactate $^-$] alone. Changes in muscle [SID] account for the majority of the change in muscle $[\text{H}^+]$ (Fig. 6.2.4).

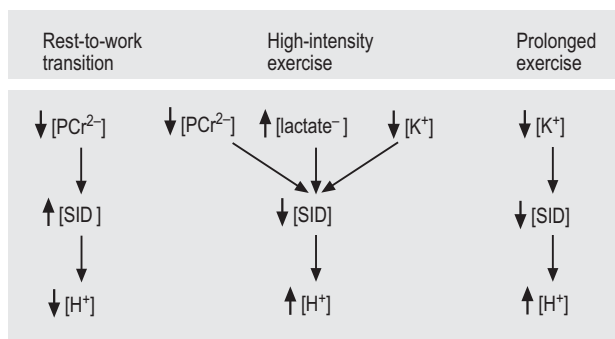


Fig. 6.2.3
Factors that affect muscle [SID], and the impact on muscle $[\text{H}^+]$, during exercise.

Muscle $[\text{A}_{\text{tot}}]$ during exercise

Exercise affects the intracellular protein portion of $[\text{A}_{\text{tot}}]$ (which has units of concentration) primarily

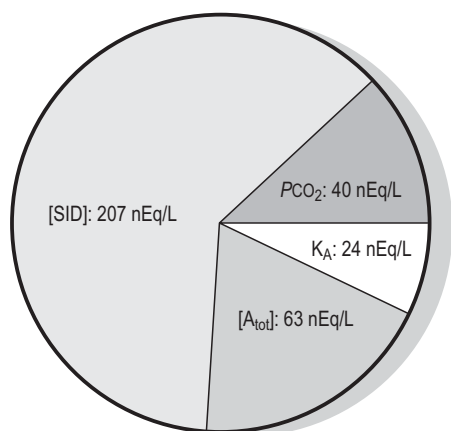


Fig. 6.2.4

Effects of changes in independent physicochemical variables on muscle $[H^+]$ after high-intensity exercise. The example provided is a brief period of very high-intensity exercise resulting in a decrease in muscle pH from 7.00 to 6.48, equal to an increase in $[H^+]$ from 100 to 334 nEq/L. (Data from Lindinger.⁶)

through changes in cell volume or intracellular water content.⁷² These volume changes are pronounced during the first several minutes of exercise⁷³ and also much later during prolonged exercise resulting in intracellular dehydration. The impact of cell volume changes on skeletal muscle $[A_{tot}]$ and acid–base state has not been studied, although it appears that the total capacity to buffer protons may not be appreciably affected because the content of carnosine remains constant.⁷⁸

With high-intensity exercise, a number of the minor variables contribute to changes in $[A_{tot}]$, including an increase in [creatine] resulting from PCr^{2-} hydrolysis, a decrease in [ATP], and an increase in [Pi]. The increase in [creatine] results from PCr^{2-} hydrolysis, while increases in Pi are due to ATP hydrolysis, increases in glycolytic phosphates, and decreases in ATP.⁴⁶ Taken together, the increases in [creatine] and [Pi] account for all of the increase in $[A_{tot}]$ during high-intensity contractions in rat skeletal muscle.⁴⁶ An increase in $[H^+]$ does not affect $[A_{tot}]$, but does decrease $[A^-]$ and increase $[HA]$, because H^+ is buffered by this non-bicarbonate buffer system. Because the concentrations of the constituents comprising $[A_{tot}]$ change during exercise, the K_A also changes. The increases in $[A_{tot}]$ and K_A accounted for 19% and 7%, respectively, of the increase in $[H^+]$ during 5 min of

high-intensity exercise in rat muscle;⁶ these have yet to be determined in equine muscle.

Muscle CO₂ during exercise

During high-intensity exercise CO₂ is produced primarily as a result of H^+ buffering by HCO_3^- , with a minor although increasing amount from tricarboxylic acid (TCA) cycle activity. Within the TCA cycle, the dehydrogenation of oxalosuccinate to α -ketoglutarate and of α -ketoglutarate to succinyl-CoA produces one molecule of CO₂ for each carbon entering the cycle.

Muscle $[H^+]$ responds in near-linear manner to increases in intracellular PCO_2 , with a 90 mmHg increase in PCO_2 (40 to 130 mmHg) increasing $[H^+]$ from 100 to 140 nEq/L.⁶ Therefore, increases in CO₂ contribute relatively little to contraction-induced changes in intracellular $[H^+]$, compared to the relatively large effect of CO₂ on plasma $[H^+]$ (Fig. 6.2.4). The contribution of CO₂ to intracellular acid–base state during exercise can only be estimated using venous plasma PCO_2 in blood draining intensely contracting muscles; this estimate assumes that intracellular muscle PCO_2 is similar to muscle venous PCO_2 .⁷⁹ This is reasonable given that CO₂ is highly diffusible and that there is substantial carbonic anhydrase within the interstitium, within the cells, and on the sarcolemma to catalyze the conversion of HCO_3^- to CO₂. It is likely that the majority of the increase in PCO_2 results from the reaction of metabolically and physicochemically produced H^+ with HCO_3^- to produce CO₂ and water.

Draught exercise

While the responses described above are typical of racing horses, very similar responses appear to occur in horses pulling heavy loads. As occurs with racing, increasing exercise intensity results in increasing recruitment of fast motor units and fast glycolytic fiber types. Standardbreds performing incremental draught-loading exercise while trotting slowly (4.8 m/s) showed a similar muscle metabolic profile⁸⁰ to that seen during racing.⁶¹ Indeed, after 10–12 min of incremental draught loading, muscle $[PCr^{2-}]$ decreased by 15 mEq/L and $[lactate^-]$ increased by 20 mEq/L, with one horse achieving a muscle $[lactate^-]$ of 57 mEq/L.⁸⁰ This study is noteworthy in that the muscle biopsies were obtained 10 s after cessation of the exercise.

Changes in plasma during exercise and recovery

As noted previously, the acid–base disturbance within the plasma during exercise is generated within contracting skeletal muscle. It must therefore be appreciated that the venous plasma draining contracting muscles displays the greatest and most rapid changes in metabolite, electrolyte, and gas concentrations compared to mixed venous or arterial plasma. The changes in mixed venous plasma will be greater than those in arterial plasma, and those in arterial plasma will be greater than those seen in venous plasma draining non-contracting tissues (i.e. jugular vein).^{81–83}

These differences in blood sampling site are important considerations when evaluating the acid–base and electrolyte profile of exercised horses. In laboratory conditions, acid–base determinations are best performed on arterial and on venous plasma draining contracting skeletal muscle. While blood samples have been collected from the iliac vein in resting horses,⁸⁴ the technique does not yet appear to have been applied to exercising horses. The only real difference in acid–base composition between mixed venous and arterial plasma is due to CO_2 loss at the lung but this, together with associated measures of PO_2 , provides valuable information on the respiratory system during exercise and recovery.¹³ When an acid–base assessment is performed on jugular venous plasma (as it often must be due to ethical or field considerations), then it must be kept in mind that a less severe and somewhat erroneous picture of whole body acid–base status will be the result.

As with muscle, it must also be appreciated that the rapid loss of water and some electrolytes from the plasma compartment with the onset of exercise is among the factors that produce changes in measured concentrations of electrolytes, metabolites, protein, and red cells (hematocrit or packed cell volume; PCV).^{85,86} Indeed, about 50% of the increase in plasma $[\text{K}^+]$, all of the increase in [plasma protein], and 50% of the increase in PCV is due to loss of water from the plasma compartment.^{85,86} The remainder of the increase in plasma $[\text{K}^+]$ is due to net loss of K^+ from contracting skeletal muscle, and the remaining increase in PCV is due to splenic contraction resulting in discharge of red cells into the circulation.

Assessment of acid–base status in the blood is performed on constituents measured within the plasma compartment. Therefore concentrations of metabo-

lites measured on samples of lysed whole blood cannot be used because the concentration of these substances within red cells differs from that in plasma. The differences between plasma and red cell intracellular concentrations diminish within the syringe after blood sampling.⁸⁷ It is therefore important to separate red cells from plasma immediately after sampling the blood from exercising horses so that the plasma sample is as close to a true reflection of what was in the plasma of the horse at the exact time of blood sampling. As noted above, the PCO_2 at different blood sampling sites varies considerably and is an important consideration when determining whole-animal acid–base status, and some of this can be due to marked temperature differences. Determination of the actual pH, PCO_2 , and PO_2 (as opposed to that measured by an instrument at 37°C), and hence $[\text{HCO}_3^-]$ requires a non-linear correction of the blood gas and pH values to the temperature within each blood sampling site.^{82,88} Increases in temperature result in increases in PCO_2 , PO_2 , and $[\text{H}^+]$ (decreased pH).

As in contracting muscle, the main changes that affect plasma [SID] during moderate- to high-intensity exercise are increases in $[\text{K}^+]$, $[\text{lactate}^-]$ ⁷⁷, and $[\text{Na}^+]$ but not $[\text{Cl}^-]$ ^{81,82} due to the greater rate of loss of water (than of Na^+) from plasma into contracting muscle. Examples of these types of exercise include incremental exercise to fatigue, maximal intensity sprints, and constant rate submaximal exercise tests.

The arterial PCO_2 responses to exercise are highly dependent on running velocity and, during incremental exercise tests, on the duration of time spent at each running velocity. Arterial PCO_2 remains unchanged during low- to mild-intensity exercise. During incremental exercise to fatigue, arterial PCO_2 decreased slightly but mixed venous PCO_2 increased markedly from 50 mmHg at rest to 80–95 mmHg at the highest work load, corresponding to 100% $\dot{V}\text{O}_{2\text{peak}}$.⁸¹ In Fenger et al's study⁸¹ running velocity was increased in small increments (0.5 to 1 m/s) and each velocity sustained for 90 s to achieve near-steady-state of cardiovascular and respiratory responses.⁸⁹ When velocity was consistently increased at 0.5 m/s increments with 4 min at each speed, increasing exercise duration was associated with a marked and progressive hypocapnia⁸² indicative of increasing alveolar ventilation.⁹⁰ In contrast, when velocity is increased in large (2 m/s) increments with only 1 min at each speed^{91,92} or when single high-intensity (running at >10 m/s) exercise bouts are performed the increase in PCO_2 is similar to that achieved during high-intensity sprint exercise.^{91,93} In horses sprinting at 115% of peak $\dot{V}\text{O}_2$, arterial PCO_2

increased from 42 mmHg at the walk to 48 and 59 mmHg at 45 and 75 s of the sprint, comparable to the 58 mmHg PCO_2 seen with rapid, non-steady-state incremental exercise test.⁹¹ From these and other similar results it can be concluded that sprinting or rapid increases in exercise intensity result in elevations in arterial PCO_2 , indicating that the respiratory system cannot keep the pace of metabolic and physicochemical CO_2 production. However, if sufficient time (90 s) is allowed at each running velocity, the rate of CO_2 elimination by the respiratory system meets or exceeds the rate of CO_2 production.

Incremental steady-state exercise

With this overview, let us now conduct an acid–base evaluation during the incremental ‘steady-state’ exercise test of Fenger et al.⁸¹ The reason for choosing this type of test is the fact that it is a very good design for the controlled, clinical assessment of respiratory and metabolic systems during and following exercise. Although the $[A_{tot}]$ data are incomplete, this particular study provides a reasonably good assessment of acid–base state using arterial plasma.

In arterial plasma, the dependent variable $[H^+]$ increased by 21 nEq/L at maximal exercise, and $[HCO_3^-]$ decreased from 32.8 to 16.2 mEq/L. The independent variable $[SID]$ remained unchanged (36.8 at rest and 35.5 mEq/L at maximal exercise) and thus had no effect on the changes in $[H^+]$ or $[HCO_3^-]$. Arterial PCO_2 decreased from 44 at rest to 35.5 mmHg at maximal exercise; this reduction appeared to be due to an increase in alveolar ventilation during the progressive exercise test and effectively reduced the mixed venous PCO_2 of 83 mmHg to below resting values. By solving for $[H^+]$ with this decrease

in PCO_2 , but holding $[SID]$ and $[A_{tot}]$ constant at resting values, the decrease in PCO_2 alone (independent of any other changes) contributed to a 7.7 nEq/L decrease in $[H^+]$, indicative of considerable sensitivity of $[H^+]$ to changes in PCO_2 . Because this decrease in PCO_2 caused a decrease in $[H^+]$, it means that an increased $[A_{tot}]$ and/or a decreased $[SID]$ had to contribute to the increased $[H^+]$ (acidosis). The numerical (although not statistically significant) 1.3 mEq/L decrease in $[SID]$ accounts for only 5.3 nEq/L of the 21 nEq/L increase in $[H^+]$. Therefore, the increase in $[A_{tot}]$ accounts for the difference between measured change in $[H^+]$ and the decrease in $[H^+]$ due to decreased PCO_2 , as well as the difference between the measured change $[H^+]$ and the increase in $[H^+]$ due to the increase in $[A_{tot}]$ (Table 6.2.4). It may appear unusual that $[SID]$ was unchanged at maximal exercise, compared to rest, so we will examine this in more detail. As expected, the main contributor to a decrease in $[SID]$ during exercise is an increase in $[lactate^-]$, and indeed lactate increased to 17 mEq/L at maximal exercise; the 16 mEq/L increase in $[lactate^-]$ thus contributed to a 16 mEq/L decrease in $[SID]$. This effect of increased $[lactate^-]$ (from 1 to 17 mEq/L), however, was offset by a simultaneous 11 mEq/L increase in $[Na^+]$ and a 3 mEq/L increase in $[K^+]$, with no change in $[Cl^-]$.

The important point about arterial acid–base balance during this type of exercise is that the increases in plasma total weak acids (primarily albumin) and inorganic phosphate are the primary contributors to the acidosis. While the 16 mEq/L increase in the strong acid anion $[lactate^-]$ did have a pronounced acidifying effect (the increase in $[lactate^-]$ alone effectively increased $[H^+]$ by ~55 nEq/L), this effect is offset by the increases in plasma strong base cations $[Na^+]$ and $[K^+]$. The increase in $[Na^+]$ results from the net movement of a low $[Na^+]$ plasma filtrate into skeletal

Table 6.2.4 Contributions to increases in arterial plasma-dependent variables $[H^+]$ and $[HCO_3^-]$ by each of the independent variables $[SID]$, PCO_2 , and $[A_{tot}]$ during incremental ‘steady-state’ exercise in horses, using data from reference⁸⁰

Variable	Resting	Maximal exercise	Change from rest	Contribution to change in $[H^+]$ (mEq/L)
$[H^+]$ mEq/L (pH)	33 (7.47)	54 (7.26)	+21	–
$[HCO_3^-]$ mEq/L	32.8	16.2	–16.6	–
$[SID]$ mEq/L	36.8	35.5	–1.3	+5.3
PCO_2 mmHg	44.0	35.5	8.5	–7.7
$[A_{tot}]$ mmol/L	Not given	Not given		+23.4*

*Calculated as $[H^+]$ change from rest (21) – ($[SID]$ and PCO_2 effects).

muscle while the increase in $[K^+]$ arises from net K^+ loss by contracting skeletal muscle.^{73,75} Finally, within mixed venous plasma the increases in $[A_{tot}]$ and PCO_2 both contribute substantially to the acidosis, and this PCO_2 effect is abolished upon transit of the blood through the lungs.

High-intensity sprint exercise

The second example to be illustrated is using high-intensity sprint exercise. We will specifically examine the second and ninth sprints of a series of nine sprints performed by trained Arabian horses, as described by Kronfeld and colleagues.⁹³ Each sprint lasted for 60 s, with a 4-min active recovery between sprints. After a warm-up, the first sprint was performed at 7 m/s (6% incline) and the remaining eight sprints at 10 m/s; blood was sampled from the jugular vein.

At the end of sprint 2, as in Fenger et al's study,⁸¹ there was only a small 1 mEq/L decrease in $[SID]$ that theoretically contributed 1 nEq/L to the 5 nEq/L increase in $[H^+]$ (Tables 6.2.5 and 6.2.6). In these tables, this is reported as -0.6 nEq/L because this more accurately represents the mean of the individual calculations for eight horses. The point is that changes in $[SID]$ are small and do not play a major role in acid-base balance during this type of exercise.

Although a 4.3 mEq/L increase in plasma $[lactate^-]$ contributed to an acidifying effect, this was offset by increases in plasma $[Na^+]$ (1.7 mEq/L) and $[K^+]$ (1.4 mEq/L). As the number of sequential sprints increased, plasma $[lactate^-]$ continued to increase to 9.5 mEq/L at the end of sprint 9; this now offset the increases in $[Na^+]$ and $[K^+]$, resulting in a 3.6 mEq/L decrease in $[SID]$ that could alone account for all of the increase (3.8 nEq/L) increase in $[H^+]$.

PCO_2 peaked at the end of the second sprint and then decreased progressively to below resting by the end of sprint 6. This indicates both a lowering of glycolytic metabolism and resultant decrease in metabolic H^+ production^{60,68} that reduces the generation of PCO_2 (from H^+ combining with HCO_3^-) and an improvement in alveolar ventilation as the exercise progresses. None the less, the decrease in PCO_2 to below resting values contributed to an alkalinizing effect equivalent to reducing $[H^+]$ by 2.4 nEq/L. A further small increase in $[A_{tot}]$ also contributed to an acidifying affect that offset most of the alkalinizing effect of lowered PCO_2 .

In summary, comparing the results of sprints 2 and 9, we see a marked difference in the origins of the acid-base disturbance. The primary acidifying influence at the end of sprint 2 was the increase in PCO_2 , whereas at the end of sprint 9 PCO_2 had decreased and contributed to an alkalinizing effect. This is an important regulatory aspect, for this later alkalinizing effect

Table 6.2.5 Contributions to increases in jugular venous plasma-dependent variables $[H^+]$ and $[HCO_3^-]$ by the independent variables $[SID]$, PCO_2 , and $[A_{tot}]$ at the end of the second sprint, using data from Kronfeld et al⁹³

Variable	Resting	Second sprint	Change from rest	Contribution to change in $[H^+]$ (nEq/L)
$[H^+]$ nEq/L	38.7	43.7	5.0	—
$[SID]$ mEq/L	48.6	47.6	-1.0	-0.6
PCO_2 mmHg	51.6	57.5	+5.9	4.1
$[A_{tot}]$ mmol/L	18.5	19.5	+1.0	1.5

Table 6.2.6 Contributions to increases in jugular venous plasma-dependent variables $[H^+]$ and $[HCO_3^-]$ by the independent variables $[SID]$, PCO_2 , and $[A_{tot}]$ at the end of the ninth sprint, using data from Kronfeld et al⁹³

Variable	Resting	Ninth sprint	Change from rest	Contribution to change in $[H^+]$ (nEq/L)
$[H^+]$ nEq/L	38.7	42.1	+3.8	—
$[SID]$ mEq/L	48.6	45	-3.6	+4.4
PCO_2 mmHg	51.6	48.2	-3.4	-2.4
$[A_{tot}]$ mmol/L	18.5	20	+1.5	+2.0

of lowered PCO_2 markedly reduced the acidifying effects of decreased [SID] (which account for two-thirds of the acidification) and increased $[A_{tot}]$ (one-third of the acidification at the end of sprint 9). In sprint 2, there was negligible effect of [SID] as $[lactate^-]$ continued to increase in plasma with repeated sprints and a progressively decreasing [SID] played an increasing role in systemic acidification. In classical acid–base physiology, one can refer to this acidosis at the end of sprint 2 as a primary respiratory acidosis that progresses to a primary metabolic acidosis with respiratory compensation by the end of sprint 9.

It should be noted that repeated sprints result in a sequential lowering of $Paco_2$ with each successive sprint⁹³ and that this is attributed to a significant increase in alveolar ventilation and not to increased breathing frequency, which is tightly coupled to stride frequency.⁹⁰ After 5 sprints, an increasing arterial hypocapnia was observed with each successive sprint.⁹³ In a similar vein, when a low-intensity exercise warm-up precedes a bout of high-intensity exercise, there is an increase in $\dot{V}CO_2$ during the first minute of exercise compared to when no warm-up preceded the high-intensity exercise.^{94,95} Rather than evoking an increase in alveolar ventilation to explain this result, however, these authors suggested that the warm-up resulted in increased tissue CO_2 storage, which then reduced the amount of CO_2 released during the subsequent bout of high-intensity exercise; this does not preclude an increase in alveolar ventilation after a warm-up. Furthermore, since the increase in $\dot{V}CO_2$ occurred within seconds of starting the high-intensity exercise,⁹⁴ it is unlikely that the rapidity of the augmented $\dot{V}CO_2$ response could be explained by a reduced ability to store CO_2 . It can be concluded that the augmented $\dot{V}CO_2$ during high-intensity exercise after warm-up is primarily due to increased alveolar ventilation, similar to that seen with high-intensity steady-state exercise. In further support, during heavy exercise (4.5 m/s at a 10% grade = 60% of peak $\dot{V}CO_2$) of 30 min duration, a progressive decrease in $Paco_2$ was primarily due to a progressive increase in alveolar ventilation secondary to increases in both respiratory frequency and tidal volume.⁹⁶

The magnitude and time course of changes in parameters describing acid–base status during recovery from exercise have not been extensively studied. It has recently been shown that recovery from the acidosis of high-intensity exercise is primarily the result of increasing [SID] (due to metabolic removal of $[lactate^-]$) and decreases in $[A_{tot}]$ (due to recovery of plasma volume)⁹⁷. By 20 min after cessation of exer-

cise plasma $[H^+]$ was not different from pre-exercise, and by 90 min of recovery it was significantly lower than pre-exercise. Full recovery of all dependent and independent acid–base variables required 60 minutes, and further recovery (90–120 minutes) was associated with a mild alkalosis with elevated $[TCO_2]$.

Steady-state submaximal exercise

The final example exemplifies the changes and contributions seen during steady-state, submaximal exercise in well-trained horses. A time point of 15 min into exercise was selected because this is well past the rapid changes that occur with the onset of exercise and was at least 10 min prior to the onset of fatigue for this intensity with this group of horses (Lindinger MI et al, unpublished data). One of the most noteworthy features of submaximal steady-state exercise in trained horses is the alkalosis that occurs during the steady-state period (Table 6.2.7). Plasma pH rose in both arterial (carotid artery) and mixed venous (pulmonary artery). In arterial plasma, the alkalosis was completely due to the decrease in $Paco_2$, since there was negligible change in [SID] (2 mEq/L increase in $[lactate^-]$ balanced by 1 mEq/L decrease in $[Cl^-]$ and 1.3 mEq/L increase in $[K^+]$) and a 1.3 mEq/L increase in $[A_{tot}]$ had an acidifying effect. The decrease in arterial plasma $[HCO_3^-]$ is a hallmark of ‘metabolic acidosis’ in the clinical acid–base sense and, indeed, that is what one would expect to see with exercise. Importantly, however, this decrease is primarily due to the increase in $[A_{tot}]$ (primarily plasma proteins) and secondary to the decrease in $Paco_2$ (despite the marked 14.4 mmHg decrease). It must be emphasized that such modest increases in $[K^+]$ and $[lactate^-]$, with the alveolar hyperventilation, are features of well-trained horses.

In mixed venous plasma, there was a small decrease in $PmvCO_2$, indicating that the influence of alveolar hypoventilation persisted within the peripheral circulation and that the rates of metabolic H^+ production were low; metabolism was primarily aerobic, as evidenced by the low plasma $[lactate^-]$ of 3.25 mEq/L. The sole contributor to an acidifying effect was the increase in plasma [proteins] ([PP]), resulting from the fluid shift into contracting muscle. The 4 mEq/L increase in [SID] thus accounted for all of the alkalizing effect. Plasma [SID] increased due to a 2 mEq/L increase in $[Na^+]$, a 1.4 mEq/L increase in $[K^+]$, and a 2 mEq/L decrease in $[Cl^-]$ that offset the 1.9 mEq/L rise in $[lactate^-]$. Mixed venous plasma $[HCO_3^-]$ increased

Table 6.2.7 Contribution to increases in arterial and mixed venous—dependent variables $[H^+]$ and $[HCO_3^-]$ by the independent variables $[SID]$, PCO_2 , and $[A_{tot}]$ at 15 min of steady-state exercise prior to onset of fatigue

Variable	Resting	Exercise	Change from rest	Contribution to change in $[H^+]$	Contribution to change in $[HCO_3^-]$
Arterial plasma					
$[H^+]$ nEq/L (pH)	37 (7.43)	26 (7.58)	−10	—	—
$[HCO_3^-]$ mEq/L	28.3	26.3	−2.0	—	—
$[SID]$ mEq/L	39.9	39.6	−0.3	+0.4	−0.3
PCO_2 mmHg	42.7	28.3	14.4	−12.0	−0.6
$[A_{tot}]$ mmol/L	13.5	14.8	+1.3	+1.4	−1.0
Mixed venous plasma					
$[H^+]$ nEq/L (pH)	43 (7.37)	38 (7.42)	−5	—	—
$[HCO_3^-]$ mEq/L	28.4	31.0	+2.6	—	—
$[SID]$ mEq/L	39.9	43.8	+3.9	−4.8	+3.7
PCO_2 mmHg	49.2	48.1	−1.1	−0.9	0.0
$[A_{tot}]$ mmol/L	13.6	14.9	+1.3	+1.6	−1.0

Data represent averages obtained from four trained Standardbreds (from Lindinger MI et al, unpublished).

during steady-state exercise, in contrast to what was seen in arterial plasma and what would also be seen in jugular venous plasma. Again, this is solely due to the increase in $[SID]$, as the increase in $[A_{tot}]$ had the effect of reducing $[HCO_3^-]$ by 1 mEq/L.

It is evident from this analysis, using relatively small changes in both dependent and independent acid–base variables, that $[H^+]$ has similar sensitivities to physiological changes in the independent variables; however, $[HCO_3^-]$ is much more sensitive to changes in $[SID]$ and $[A_{tot}]$ than to changes in PCO_2 .

Endurance exercise

Low-speed endurance exercise, such as draught horses pulling loads for 8 h per day for 5 consecutive days,⁹⁸ produces no significant acid–base disturbances. When the speed of endurance exercise is increased, with ensuing sweat losses of water and electrolytes occurring at elevated rates, then a slowly progressing metabolic alkalosis develops.^{99–101}

The metabolic alkalosis associated with dehydration during endurance rides, or indeed during prolonged transport of a horse, is a direct result of sweating. Equine sweat has a tonicity similar to that of plasma¹⁰² in contrast to the very dilute sweat produced by humans. The reason for the high tonicity of equine sweat is that the equine sweat gland does not appear to have the ability to resorb ions from within the lumen of the sweat gland. Rather, some ions, such as K^+ and Cl^- , appear to be secreted for their concentrations in sweat are much greater than in plasma and

extracellular fluid.¹⁰² Although the electrolyte composition of equine sweat changes over time, in general, sweat $[Na^+]$ ranges from 65 to 170, $[K^+]$ ranges from 25 to 55, and charge balance is made up by $[Cl^-]$ in the range 100–180 mEq/L.¹⁰² The total amount of electrolyte loss during a 100-mile endurance ride can be in excess of 1 mole for Na^+ and Cl^- , representing substantial depletion of body stores,¹⁰³ so it is instructive to determine the origins of the electrolyte losses. Because the tonicity and $[Na^+]$ of equine plasma and sweat are similar, there is little if any change in plasma osmolality and $[Na^+]$ during prolonged endurance exercise.^{70,103,104} Additionally, when plasma $[K^+]$ is measured, after allowing sufficient time after exercise has stopped for re-equilibration of plasma and muscle K^+ pools, there is little change in plasma $[K^+]$ and clearly the K^+ losses are not borne by the extracellular compartment. Therefore, the K^+ appearing in the sweat originates largely from cells, and likely contracting muscle cells that are known to lose K^+ during the period of exercise.¹⁰⁵ It is important to recall that the bulk of the body's Na^+ and Cl^- stores are extracellular, and it is Cl^- that balances the positive charge on Na^+ and K^+ lost in sweat. Therefore sweat $[Cl^-] = [Na^+] + [K^+]$. Because the extracellular losses of Cl^- exceed those of Na^+ by a factor ranging between 1.3 and 1.8, extracellular and plasma $[Cl^-]$ must decrease, and indeed do so by 10 to 15 mEq/L.^{70,99,103,104} It is this large decrease in plasma $[Cl^-]$ that is responsible for the alkalosis, for this results in a large increase in plasma $[SID]$.

Using the principles of acid–base assessment provided above, an extreme case of plasma $[Cl^-]$ depletion

(-15 mEq/L^{104}) will be used to exemplify the effect on acid–base status:

$$[\text{SID}] = ([\text{Na}^+] + [\text{K}^+]) - ([\text{Cl}^-] + [\text{lactate}^-])$$

Before endurance exercise:

$$38 \text{ mEq/L} = (135 + 4) - (100 + 1)$$

After endurance exercise:

$$53 \text{ mEq/L} = (135 + 4) - (85 + 1)$$

The increase in [SID] alone has a large alkalinizing effect and can be calculated to decrease plasma $[\text{H}^+]$ from 36.6 to 23.9 nEq/L (pH increase from 7.436 to 7.622), with $[\text{HCO}_3^-]$ increasing from 26.9 to 41.2 mEq/L. The fact that such large changes do not occur is primarily attributed to the simultaneous increase in plasma $[\text{A}_{\text{tot}}]$ resulting from the loss of extracellular water which raises [PP]. An increase in [PP] from 60 to 75 g/L, typical of endurance rides, increases $[\text{A}_{\text{tot}}]$ from 13 to 16.25 mmol/L, and this change alone (independent of change in [SID] or PCO_2) has the effect of raising $[\text{H}^+]$ from 36.6 to 40.5 nEq/L. The combined effect of increased $[\text{A}_{\text{tot}}]$ and decreased [SID] results in an $[\text{H}^+]$ of 25.6 nEq/L (pH 7.591) and $[\text{HCO}_3^-]$ of 38.4 mEq/L. There is negligible change in PCO_2 during endurance exercise and the small changes that occur can be neglected for the purposes of assessing acid–base status. Therefore, the acidification resulting from the increase in plasma $[\text{A}_{\text{tot}}]$ only partially offsets the alkalinization caused by the increase in [SID], resulting in the observed alkalosis.

It is very important to note that, in the dehydrated horse, there is little change in plasma $[\text{Na}^+]$ and osmolality. Horses can lose 30 L or more of fluid as sweat with minimal alteration of plasma osmolality and $[\text{Na}^+]$. This is solely because sweat osmolality and $[\text{Na}^+]$ differ little from that of sweat. To complicate the interpretation, the decrease in plasma $[\text{Cl}^-]$ is seemingly inconsistent with dehydration. The key hematological variables for diagnosis of dehydration in the horse are a decrease in plasma $[\text{Cl}^-]$ coupled with increases in [PP] and PCV.

It is important to point out that when the hydration status of the horse is maintained, through the use of effective strategies of electrolyte supplementation, an alkalosis does not develop. A detailed assessment of acid–base status in electrolyte-supplemented horses during the time course of an endurance ride was performed by Hess et al.¹⁰⁶ It was determined that oral electrolyte supplementation attenuated a decrease in plasma $[\text{H}^+]$ and actually resulted in an increased $[\text{H}^+]$ during the final phase of the ride. The increased $[\text{H}^+]$

was primarily a result of a decreased [SID], due to maintenance of baseline $[\text{Cl}^-]$ and decreases in $[\text{Na}^+]$. Interestingly, the authors determined that when a K^+ -free electrolyte solution was given, plasma $[\text{H}^+]$ was significantly lower throughout the ride compared with when a typical K^+ -rich supplement was administered. This was thought to be due to the higher Na^+ content of the K^+ -free formula.

Other types of exercise

It is worth noting some interesting features of acid–base balance reported in the literature in horses performing different types of activity, ranging from draught work to show jumping. Show jumping competition resulted in a decrease in jugular venous PCO_2 , once again illustrating the degree of alveolar ventilation and an ability of lowered PCO_2 to offset the acidifying effects due to increases in $[\text{A}_{\text{tot}}]$.¹⁰⁷ In these horses [SID] increased by 2 mEq/L, which also had an alkalinizing effect; a 2 mEq/L decrease in $[\text{Cl}^-]$ and 3 mEq/L increase in $[\text{Na}^+]$ more than offset the 3.8 mEq/L increase in $[\text{lactate}^-]$.

The second day of three-day eventing consists of a steeplechase, a roads and tracks phase, and a cross-country phase, with each separated by a rest/cool-down period. Rose and colleagues^{108,109} assessed the acid–base changes occurring within jugular venous blood during this period. As with the other forms of exercise described above, plasma $[\text{H}^+]$ decreased from 42 to 37 nEq/L at the end of the roads and tracks phase, and was subsequently 41 nEq/L at the end of the cross-country phase. The main reason for the decrease in $[\text{H}^+]$ at the end of roads and tracks was a 6 mmHg decrease in PCO_2 with a 2.3 mEq/L increase in [SID]. The increase in [SID] resulted from increases in $[\text{Na}^+]$ and $[\text{K}^+]$ and decreases in $[\text{Cl}^-]$ that offset the 1.5 mEq/L increase in $[\text{lactate}^-]$. Together, the combined effects of lowered PCO_2 and increased [SID] more than offset the acidifying effects of the 1.2 mmol/L increase in $[\text{A}_{\text{tot}}]$, producing the mild alkalosis. At the end of the cross-country, plasma $[\text{lactate}^-]$ had risen to 8.2 mEq/L, negating the effects of increased $[\text{Na}^+]$ and lowered $[\text{Cl}^-]$ and leaving [SID] at resting values (40 mEq/L). The sole contributor to an acidifying effect was thus the 3 mmol/L increase in $[\text{A}_{\text{tot}}]$ (resulting from dehydration (57%) and exercise-induced fluid shift (43%)) and this was completely offset by a further decrease in PCO_2 to 31.5 mmHg resulting in a normal $[\text{H}^+]$ (41.3 nEq/L). As expected, the combined effects of increased $[\text{A}_{\text{tot}}]$ and reduced PCO_2 lowered

$[\text{HCO}_3^-]$ from 28 mEq/L at rest and at the end of roads and tracks to 18 mEq/L at the end of the cross-country. Within 30 min of completing the cross-country phase, both $[\text{H}^+]$ and $[\text{HCO}_3^-]$ had normalized to pre-exercise values despite an elevated $[\text{A}_{\text{tot}}]$ (at this point due solely to dehydration), whose acidifying effects were offset by a continued depression of PCO_2 . In contrast to the Rose et al studies,^{108,109} when the steeplechase and roads and tracks phases were eliminated and only the cross-country phase was performed, a mild metabolic acidosis (a 3–6 nEq/l increase in jugular venous $[\text{H}^+]$) with respiratory compensation occurred that became more severe with increasing competition difficulty levels.¹¹⁰

Polo consists of periods of low to moderate activity interspersed with brief periods of burst activity while carrying the rider. The total duration of activity for individual horses ranges from 1 to 2 h, allowing time for dehydration to occur as a result of sweating (see the 'Endurance exercise' section). Polo exercise results in a modest acidosis of metabolic origin (increases in plasma $[\text{lactate}^-]$) that lasts during the period of activity.¹¹¹ After cessation of exercise a mild alkalosis may develop, concomitant with decreased plasma $[\text{Cl}^-]$ resulting from sweat losses (see 'Endurance exercise').

Clinical notes

In a clinical vein, the nature of the generation of the acid–base disturbance, and its subsequent regulation during and following the period of exercise, result in its eventual amelioration over a period of minutes to hours, depending on the intensity and duration. Very high-intensity exercise is capable of lowering arterial plasma pH to 7.1.⁷⁷ Therefore caution may be required with repeated sprints of very high intensity due to the cumulative effects of increasing $[\text{lactate}^-]$ and $[\text{PP}]$ that may be capable of decreasing arterial pH below 7.1. It must be understood that the severity of the mixed venous acidosis would be measurably greater than the arterial acidosis. At somewhat lower intensity, horses are capable of several repeated sprints without incurring a severe acid–base disturbance.⁹³ The final caution pertains to dehydration that results from prolonged endurance exercise or prolonged transport without adequate intake of fluids and electrolytes. Dehydrated horses typically have an alkalosis that results in large part from the decrease in plasma $[\text{Cl}^-]$ (loss of strong acid anion). The decrease in plasma

$[\text{Cl}^-]$ is primarily due to the very large losses of Cl^- in the sweat that serve to balance physicochemically the positive charge of Na^+ and K^+ lost in sweat. At the same time, $[\text{PP}]$ may be in excess of 8 g/dL (normal is 5–6 g/dL in a euhydrated horse) and this will have a pronounced acidifying effect that also lowers $[\text{HCO}_3^-]$. Proper correction of this combined acid–base disturbance requires oral (preferably) or intravenous (if necessary) administration of a balanced electrolyte solution to replace the water, Na^+ , K^+ , and Cl^- lost in sweat. Since Cl^- in the administered solution will balance the sum of Na^+ and K^+ , as K^+ is taken up by the tissues (and this occurs very rapidly¹²) the Cl^- will be left in the extracellular compartment with Na^+ , serving to retain extracellular fluid and thereby lower plasma $[\text{protein}]$. This effectively corrects both the strong ion and weak ion aspects of the acid–base disturbance simultaneously.

Exercise summary

The strength of the physicochemical approach to acid–base balance is that it allows for the determination of each of the known and measured variables that determines the concentrations of H^+ , HCO_3^- , and OH^- in body fluids. Specifically, the method allows us to determine how much of an effect the increase in $[\text{PP}]$ has. In most types of exercise, and in many clinical conditions, $[\text{PP}]$ is elevated; large increases in $[\text{PP}]$ are a hallmark of dehydration in endurance horses and, with other causes of dehydration, has a marked acidifying effect on plasma, and additionally lowers $[\text{HCO}_3^-]$. With high-intensity exercise, increases in plasma $[\text{lactate}^-]$ play a major role in decreasing $[\text{SID}]$, and hence acidifying the plasma and lowering $[\text{HCO}_3^-]$. With submaximal exercise, however, changes in strong ion concentrations are small and their effects on acid–base balance may be less than those exerted by increases in $[\text{A}_{\text{tot}}]$. Finally, the very large increases in mixed venous PCO_2 that occur with high-intensity exercise have a major acidifying effect, but raise $[\text{HCO}_3^-]$. When this blood is treated by the lungs, the PCO_2 is markedly lowered, often to below resting values for PaCO_2 . Thus the acidifying effect of PCO_2 within the venous plasma may be reversed to an alkalinizing effect within the arterial plasma. The main strong ion changes contributing to the decrease in $[\text{SID}]$, and hence acidification and lowering of $[\text{HCO}_3^-]$, are increases in plasma $[\text{lactate}^-]$ and decreases in plasma $[\text{Cl}^-]$.

Responses to training

The impact of training, or exercise conditioning, on acid–base balance has not been well studied. It is known, however, that the responses to exercise conditioning for low- to moderate-intensity exercise (endurance training) differ from those for high-intensity exercise (sprint training), both at the whole-body metabolic level (see Chapter 5.1) and within skeletal muscle (see Chapter 2.1). Perhaps because of relative ease of implementation, endurance-type training studies are more prevalent than sprint training studies.

Endurance training

Endurance training results in a decreased contribution of ATP production from anaerobic muscle metabolism¹¹² and an increased oxidative capacity of muscles¹¹³ that leads to an increased reliance and capacity for fatty-acid oxidation during exercise. There is a reduced conversion of pyruvate to lactate[−] accompanied by improved matching of glycolytic flux with entry of pyruvate into the TCA cycle.¹¹⁴ Notably, these adaptations result in a reduced rate of lactate[−] and H⁺ accumulation within contracting muscle and blood, and these responses may be detectable within the first week of training. This is associated with increases in the activities of enzymes of oxidative metabolism that favor an increase in free fatty acid oxidation. In highly trained, elite human endurance athletes the adaptations for fat oxidation are very pronounced, with fat serving as the main energy source at exercise intensities as high as 85% of peak $\dot{V}O_2$ ¹¹⁵ with concomitant decrease in muscle glycogen utilization at submaximal exercise intensities. At present, it is not known if the elite equine endurance athlete can achieve a similarly high capacity to utilize blood-borne fatty acids.

These intramuscular biochemical adaptations are associated with improvements in the cardiovascular function (increased stroke volume and cardiac output)⁸⁹ but there appears to be little change in functional parameters of the respiratory system.^{89,92,116} In humans performing submaximal intensity exercise, short-term endurance training appears to attenuate the exercise-induced increase in plasma [H⁺], as a result of less lactate[−] production and a greater plasma volume, leading to a greater [SID] and lower [A_{tot}].¹¹⁷ However, in horses endurance training appears to have no effect on acid–base variables during low-

intensity exercise,¹¹⁸ probably because the metabolic and cardiorespiratory systems are not sufficiently taxed by low work rates.

Sprint training

The effects of sprint training on the ability of skeletal muscle or the whole body to regulate acid–base state have not been studied. Nevertheless, a number of inferences may be made from the limited results available. Within skeletal muscle, sprint training results in a large increase in non-bicarbonate buffering capacity⁴⁴ that appears to be due primarily to increases in muscle carnosine content.⁵⁶

Seven weeks of race training in Thoroughbreds increased skeletal muscle non-bicarbonate buffering capacity by 60%, from 58 ± 7 to 93 ± 7 mmol/kgpH^{−1}.⁴⁴ Similar increases have been reported in humans^{5,119} but sometimes not in other equine studies.^{120,121} In the latter studies, however, the untrained horses were likely somewhat active, making the detection of significant differences difficult. Buffering capacity, even in untrained equine muscle, is substantially greater than in trained humans, and is 43 mmol/kgpH^{−1}.^{119,122} The increased buffering capacity with sprint training is very important in view of the fact that sprint training (in humans) results in increased activities of the glycogenolytic and glycolytic enzymes phosphorylase, phosphofructokinase, glyceraldehyde phosphate dehydrogenase, and lactate dehydrogenase.¹²³ Indeed, a high degree of correlation between carnosine content and glycogen phosphorylase activity has been shown.⁵⁰ Increased activities of these enzymes would be associated with a capacity to increase the rates of lactate[−] and H⁺ production within contracting muscle. Despite increased rates of production, there was actually a decrease in lactate accumulation within muscle after training compared to before training both in horses¹²¹ and in humans,¹¹⁹ suggesting decreased reduction of pyruvate to lactate and enhanced pyruvate conversion to acetyl-CoA. Additionally, increased amounts of lactate[−] could be transferred out of contracting muscle cells by training-induced increases in the monocarboxylate transporter MCT,¹²⁴ to be taken up rapidly by other tissues,⁴⁹ keeping plasma [lactate[−]] relatively low.¹²¹

Thoroughbred race training resulted in no effects on pH_a, PaCO₂, PmvCO₂ and $\dot{V}CO_2$ after 7 weeks⁸⁹ or 16 weeks,⁹² although arterial⁸⁹ and mixed venous pH⁹² decreased significantly less than before training during incremental exercise tests.

In summary, from the limited number of sprint training studies, it appears likely that an improved ability to regulate acid–base state with moderate- to high-intensity exercise is primarily due to increases in non-bicarbonate proton buffering capacity and an improved ability to utilize pyruvate.

High altitude

The transition to high altitude results in a number of hematological adjustments that affect acid–base status.^{125,126} The decreased atmospheric partial pressure of O₂ results in an alveolar hyperventilation that persists for at least 6 days. During the initial 3 days of transition to 3800 m elevation, *PCO*₂ decreased from 40 to 20 mmHg and [SID] from 44 to 26 mEq/L; together, these changes accounted for increases in pH (7.43 to 7.48) and decreases in [HCO₃[−]] (from 23 to 15 mEq/L).¹²⁶ After the third day of altitude, acid–base parameters were normalized over a 3–7-day period.¹²⁶ Since [PP] was not measured, the influence of [A_{tot}] on acid–base balance is unknown at this time. From these studies it appears that a minimum of 10 days is needed to complete the adjustments affecting plasma acid–base status. This is an important consideration when horses are brought from low- to high-altitude locations for competition.

Diet and acid–base

Nutrition plays an important role in equine health, performance, and acid–base balance, both at rest and during exercise. Large-animal diets are often characterized on the basis of their dietary cation–anion difference (DCAD), defined as the balance between strong base cations (K⁺ and Na⁺) and strong acid anions (Cl[−] and SO₄^{2−}). The reason that DCAD affects acid–base balance in the body is because diets high in cations but low in anions (high DCAD) result in an increased cation content of the extracellular fluids as these cations are absorbed within the small intestine. With a sustained high DCAD diet, much of the excess cation is excreted by the kidneys, but the cations are accompanied by the weak acid HCO₃[−] and the strong acid Cl[−], typically producing a mild systemic alkalosis.¹²⁷ In contrast, low DCAD diets produce a systemic acidosis^{127–129} with increased renal calcium excretion that

could lead to negative calcium balance and weakening of the skeletal system.¹³⁰ A DCAD of between 250 and 300 mEq/kg is considered neutral; this will not result in significant alterations of acid–base balance. In contrast, a DCAD >300 mEq/kg will have an alkalinizing effect, while a DCAD <250 mEq/kg will have an acidifying effect. High-quality, high-forage diets have a large cation content, primarily K⁺, that will often produce a DCAD >300 mEq/kg. In contrast, unsupplemented grain rations have a high Cl[−] and low K⁺ content and have a DCAD typically less than 250 mEq/kg. There has been considerable research over the past few decades into the chronic effects of feeding diets with varying DCAD on equine whole-body acid–base balance (for review see Waller et al¹³¹), and the key results are highlighted below.

Resting horses

Plasma pH and [HCO₃[−]], and urine pH have been shown to increase in proportion to the DCAD over the range 0–407 mEq/kg dry matter.^{127,129,130} Diets that have a high protein content are also acidogenic due to the production of SO₄^{2−} (strong acid anion) and inorganic phosphate (weak acid anion), which become elevated in plasma. In Arabian horses, a high-protein (HP) diet (DCAD=182 mEq/kg) significantly decreased [SID] by 7 mEq/L compared to horses fed on a low-protein (LP; DCAD=260 mEq/kg) diet.¹³² The decrease in [SID] was due to a 7 mEq/L increase in plasma [Cl[−]] in the HP group. It is likely that the increase in [Cl[−]] was offset by decreases in [SO₄^{2−}] and [phosphate], but these were not reported. Plasma *PCO*₂ averaged 3 mmHg higher in the HP group. The resultant changes in dependent acid–base variables was small (increased plasma [H⁺] by 2–3 nEq/L), with no effect on [HCO₃[−]].

Exercise

Only a few studies have examined the influence of DCAD on exercise performance and on acid–base balance during exercise.^{128,129,133,134} Popplewell et al¹³⁴ demonstrated that horses fed a high DCAD ran significantly faster and, at the end of exercise, had elevated concentrations of lactate[−] in the blood while plasma [H⁺] was unchanged.¹²⁹ A similar response was not seen by Cooper et al¹³³ where the high DCAD diet was marginal at 306 mEq/kg.

In the study by Graham-Thiers et al¹³² horses on the HP diet (low DCAD) had a more pronounced lowering of $[\text{HCO}_3^-]$ during exercise consisting of six sequential 1-min sprints, separated by 4-min rest periods. The lower $[\text{SID}]$ and $P\text{CO}_2$ during rest in the HP group persisted during exercise and 30 min of recovery and accounted for the reduced $[\text{HCO}_3^-]$. Of interest, and in contrast to Popplewell et al's study,¹³⁴ horses on the HP diet averaged 3 mEq/L higher plasma $[\text{lactate}^-]$, which was offset by a 3 mEq/L decrease in plasma $[\text{Cl}^-]$, than horses on the LP diet.

Dietary protein

The purported effects of dietary protein on acid–base balance in the study by Graham-Thiers et al¹³² can at least in part be attributed to the low DCAD of the HP diet. A recent study by the same group¹³⁵ maintained the DCAD of the HP diet between 387 and 424 mEq/kg dry matter, whereas the DCAD of the LP diet was 345–370 mEq/kg. At rest, horses on the LP diet had a minor lowering of arterial and mixed venous plasma $[\text{lactate}^-]$ and pH, and increased arterial $[\text{HCO}_3^-]$ and $[\text{K}^+]$, without change in other variables. With high-intensity sprinting exercise, horses on the LP diet maintained a 0.03 pH-unit higher mixed venous pH than horses on the HP diet, although the physiological significance of such a small, although consistent, effect is questionable. The increased mixed venous pH could be attributed to a 2 mEq/L greater $[\text{SID}]$ in LP horses both at rest and during exercise, with no differences in $P\text{CO}_2$ and $[\text{A}_{\text{tot}}]$ between LP and HP. In contrast, arterial plasma was not influenced by diet during exercise. These limited studies suggest that the LP diet alters the functioning of the contracting muscles sufficiently to produce a modest effect of elevated $[\text{SID}]$ and pH in mixed venous blood. Altering the amino acid composition of the diet may also affect acid–base balance. Vervuert et al¹³⁶ demonstrated that supplementing horses with 50 g of tryptophan resulted in 0.7–2.0 mmol/L lower blood $[\text{lactate}^-]$ after trotting exercise. The effects of these dietary protein/amino acid studies are so small that they remain of questionable physiological significance.

Dietary starch

Dietary starch has been suggested to be the cause of a decrease in plasma pH when DCAD was held constant across diets. However, a recent comprehensive study

using three high DCAD (305–333 mEq/kg) and three low DCAD (124–154 mEq/kg) diets, with starch comprising 45–49% of each diet, showed that high-starch diets (from corn, oats, or alfalfa) had no effect on plasma acid–base balance.¹³⁷ These authors concluded that the potential negative effects of high-grain diets (low DCAD) can be overcome by adjustment of the DCAD with supplemental cations. There was also no effect of alfalfa versus non-alfalfa roughage diets on acid–base status and exercise performance in Thoroughbreds.¹³⁸

Dietary fat

The effect of dietary fat on performance and acid–base balance has received more attention than starch and protein. Graham-Thiers et al found no effect of dietary fat (0 versus 10%) on acid–base balance at rest and during repeated sprint exercise, although there was evidence for an increased reliance on fat as an energy source.¹³⁵ In contrast, an earlier study of repeated sprint exercise reported that horses on a 10% fat diet showed an increased plasma acidosis with increased $[\text{lactate}^-]$ ¹³⁹ and decreased $P\text{CO}_2$ and $[\text{SID}]$ than horses on the control diet.¹⁴⁰ Peak effects of the high-fat diet required between 6 and 11 weeks of feeding. When horses were on a high-fat (10%) diet for a relatively short duration of 4 weeks, there was a tendency for the high-fat diet to increase plasma $[\text{lactate}^-]$ during exercise.¹⁴¹ This was associated with faster gallop speeds during the third and fourth repeated gallops, which Duren et al¹⁴¹ attributed to an increased activation of glycolysis and glycogenolysis¹⁴² with associated increases in power output with repeated sprint exercise. It appears that, with high-intensity exercise, horses must be maintained on a high-fat diet for at least 6 weeks and that the chronic high-fat diet results in an increased reliance on glycolysis to produce the ATP needed to generate the high power outputs. The effects of a high-fat diet on acid–base balance during high-intensity exercise appear to be minor. The effects of dietary fat on acid–base balance during prolonged low- and moderate-intensity exercise do not appear to have been studied.

Acute dietary effects

A recent study¹⁴³ used the physicochemical approach to characterize the acute changes in plasma acid–base state that occur in response to feeding. The feeding of

a mixed hay and grain diet resulted in rapid (within the first 1–3 h of meal consumption) changes in plasma electrolyte and acid–base variables. A post-feeding plasma acidosis (increased plasma $[H^+]$) was accompanied by a decreased $[HCO_3^-]_2$. The primary contributors to the increase in plasma $[H^+]$ were the decrease in the plasma $[SID]$ and an increase in the PCO_2 . An increase in plasma $[A_{tot}]$ post-feeding had only a minor effect on acid–base state. The feeding-induced acidosis abated 3–6 h after the meal, showing cyclical recovery of physicochemical variables that contributed to the acid–base disturbance. It is interesting to note that the time course and magnitude of feeding responses differed between the two groups of horses studied, and between the morning and evening meals, indicating that time of day and season need to be considered when interpreting equine feeding studies.

Selected clinically relevant issues for seemingly ‘normal’ horses

Age

A recent study¹⁴⁴ provided a detailed comparison of plasma acid–base status in young (<9 years) and old (>19 years) horses. The only differences were a reduced (by 1.5 mmHg) arterial PCO_2 that resulted in a lower pH (7.428 versus 7.404) and this was associated with a lower PO_2 (90 versus 102 mmHg); there were no differences in measured weak and strong ion concentrations. A similar effect of age on arterial PCO_2 has previously been reported.¹⁴⁵ Aguilera-Tejero et al suggest that the apparent hypoxemia in older horses results in an increased ventilation that would thereby reduce arterial PCO_2 .¹⁴⁴

Idiopathic laryngeal hemiplegia

A number of race horses, both Thoroughbred and Standardbred, may perform poorly due, in part, to a syndrome known as idiopathic laryngeal hemiplegia (ILH).^{145,146} ILH is a peripheral neuropathy characterized by dysfunction of the left recurrent laryngeal nerve innervating most of the left laryngeal muscles.¹⁴⁷ Haynes indicated that about 50% of large breed horses have some degree of laryngeal synchrony and asymmetry at rest.¹⁴⁸

Impaired regulation of acid–base balance occurs during exercise in horses with grade 4 or 5 laryngeal function,¹⁴⁷ where grade 5 is complete hemiplegia. The primary manifestation during a standard exercise test to fatigue is a marked elevation in arterial PCO_2 (grade 4, 69 mmHg; grade 5, 75 mmHg) compared to grade 1 (normal laryngeal function, 58 mmHg) that is associated with a reduced arterial PO_2 (by 8 mmHg) and increased (by 5–6 mEq/L) plasma $[lactate^-]$.^{146,147}

Small airway inflammation and exercise-induced pulmonary hemorrhage (EIPH) also commonly occur in race horses, leading to decrements in performance.¹⁴⁹ Poorly performing horses with these respiratory diseases manifested a pronounced and sustained hypocapnia with lower arterial PO_2 , $[HCO_3^-]$ and pH and higher blood $[lactate^-]$ during recovery from high-intensity exercise. The more pronounced increase in $[lactate^-]$ is consistent with impaired blood oxygenation at the lungs, resulting in a greater reliance on anaerobic mechanisms to produce energy during high-intensity exercise. The authors' conclusion that the hypocapnia was due to the lactic acidosis¹⁴⁹ cannot be supported. While the hypocapnia itself would blunt the arterial chemoreceptor drive to ventilate, this may be offset by the increased $[H^+]$. The hypocapnia can be explained in terms of alveolar hyperventilation, which would facilitate rapid release of CO_2 at the lungs while maintaining compromised ability to extract oxygen.

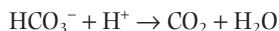
Administration of alkalinizing substances and other purported ergogenic aids

Alkalinizing substances

There is an extensive literature on the administration of bicarbonate salts and other alkalinizing agents in both horses and humans, and the interested reader is referred to reviews by Schott & Hinchcliff¹⁵⁰ and Heigenhauser & Jones.¹⁵¹ There is also evidence that the administration of alkalinizing agents will result in enhanced performance with exercise at moderate to high intensities, and that this is due to increased rates of strong acid (i.e. $lactate^-$) removal from contracting muscle. This section will focus on the physicochemical aspects of inducing and correcting the acid–base disturbance resulting from the administration of bicarbonate-, citrate-, and phosphate-containing compounds.

At the outset it must be explained that the administration of HCO_3^- does not in and of itself result in an increase in plasma $[\text{HCO}_3^-]$. When sodium bicarbonate, or baking soda, is dissolved in water, all of the Na^+ completely dissociates from HCO_3^- by virtue of Na^+ being a strong ion; by definition strong ions are completely dissociated in solution.^{23,26} The HCO_3^- in solution readily participates in physicochemical reactions with H^+ , CO_2 , and H_2O , as described above. In this simple solution consisting of water and added baking soda, the $[\text{HCO}_3^-]$ will be determined by the $[\text{Na}^+]$ and by the PCO_2 . Since this solution will come into equilibrium with the CO_2 in the air, the resulting PCO_2 will be very low once equilibrium is achieved. The addition of carbonic anhydrase to this solution would greatly increase the speed of the reaction, establishing equilibrium.

When a freshly mixed baking soda solution is administered nasogastrically into the stomach of a horse, which is an acidic environment, acid within the stomach and upper intestinal tract reacts rapidly with the HCO_3^- in solution. The HCO_3^- that was in solution rapidly dissipates, producing CO_2 and H_2O :



The CO_2 diffuses readily into the blood, where most is transformed by the action of carbonic anhydrase to HCO_3^- , transported to the lungs, reconverted to CO_2 , and eliminated in expired air. The ingested water and Na^+ are absorbed within the small intestine, resulting in increased plasma water content and $[\text{Na}^+]$; the excess is ultimately removed from the body through normal renal processes. Since the ingested HCO_3^- becomes part of the open CO_2 system, which is in a dynamic equilibrium with inspired air and the tissues, PCO_2 is rapidly normalized through ventilation. Therefore the only changes remaining within the plasma (and extracellular) compartment are increased $[\text{Na}^+]$ and decreased concentrations of other plasma ions, both strong and weak. Notably, the concentrations of the predominant weak acid, protein, and strong acid Cl^- are decreased, resulting in decreased $[\text{A}_{\text{tot}}]$ and $[\text{Cl}^-]$. Both the decrease in $[\text{Cl}^-]$ and the increase in $[\text{Na}^+]$ contribute to an increase in $[\text{SID}]$. Both the increase in $[\text{SID}]$ and the decrease in $[\text{A}_{\text{tot}}]$ contribute to increased $[\text{HCO}_3^-]$ and decreased $[\text{H}^+]$; PCO_2 remains unchanged.

Because of the nature of these physicochemical reactions, it does not matter which cation salts of bicarbonate are dissolved in water and administered to the horse; all will result in a very similar extracellular alkalosis, at least initially. If calcium salts are

used, these are absorbed slowly and the onset of the alkalosis will be slow. If a sufficient amount of calcium is used to induce an alkalosis, it is likely that this would be toxic. Potassium bicarbonate has also been used. Potassium, in contrast to Na^+ , does not remain in the extracellular compartment but is rapidly taken up by the tissues, reducing the extent of the extracellular alkalosis presumably at the expense of alkalinizing the intracellular compartment.¹²

Time course

The administration of an alkalinizing agent results in a lowering of plasma and extracellular $[\text{H}^+]$, with a concurrent increase in $[\text{HCO}_3^-]$, with peak effects appearing to depend on the volume of water administered with the dose. When 1 L of water was administered with a NaHCO_3 dose of 0.4 g/kg body mass, peak effects occurred 2–4 h after administration.¹⁵² In contrast, when 2–4 L of water was used to administer doses ranging from 0.25 to 1.5 g/kg, peak effects occurred only 6–8 h after administration.^{153–156} Similar time courses occurred with a range of alkalinizing agents (sodium citrate, sodium acetate, sodium lactate) administered at a dose of 0.5 g/kg in 2 L of water.¹⁵⁷

As noted above, the administration of an alkalinizing agent with 4 L of water should result in a transient increase in plasma volume. The time course of PV change has not been studied, although it is known that any early changes in PV that would occur are corrected 6 h after administration.¹⁵⁸ Therefore, after this 6-h period the sole cause for the acid–base disturbance is the increase in extracellular $[\text{Na}^+]$ (or other administered cation).

Mechanism for enhanced lactate efflux

The rate of lactate $^-$ -facilitated diffusion across the sarcolemma by the monocarboxylate transporter (MCT1), of which there are at least four isoforms in muscle,^{49,124} depends on the chemical gradient and driving force for H^+ and lactate $^-$. Extracellular $[\text{lactate}^-]$ is very low compared to muscle $[\text{lactate}^-]$ in horses performing moderate- to high-intensity exercise,^{159,160} providing a high driving force for lactate $^-$ diffusion out of contracting muscle cells. Similarly, extracellular $[\text{H}^+]$ is low compared to intramuscular $[\text{H}^+]$, and this is increased for a prolonged duration (up to 16 h) after administration of alkalinizing agents.¹⁵⁷ This combination of chemical changes results in an enhanced rate of removal of lactate $^-$ from contracting muscle

during high-intensity exercise, producing a higher plasma [lactate⁻] and lower muscle [lactate⁻] at the end of exercise.¹⁶⁰ In order to be able to detect these changes, the dose administered may be important; Greenhaff et al used an NaHCO₃ dose of 0.6 mg/kg,¹⁶⁰ and when a dose of 0.4 mg/kg was used no differences in muscle and blood lactate were found.¹⁵⁹

Creatine supplementation

The practice of creatine supplementation is used in humans to improve performance associated with short-term, high-intensity exercise.¹⁶¹ Attempts to replicate similar effects in horses with a dose of 25 g creatine monohydrate twice daily have shown no effect on performance, metabolism, and related acid-base variables.¹⁶²

Furosemide

Furosemide is a loop diuretic that is extensively used to treat race horses exhibiting symptoms of exercise-induced pulmonary hemorrhage (EIPH) in some racing jurisdictions.¹⁶³ There is evidence that furosemide administration improves racing performance¹⁶⁴ despite the pronounced diuresis with loss of water and electrolytes.¹⁶⁵ The mechanism by which furosemide induces a long-lasting mild alkalosis is through reductions in plasma [Cl⁻] due to marked increases in renal Cl⁻ excretion without concomitant increases in renal strong cation excretion¹⁶⁵ and movement of plasma Cl⁻ into red blood cells.¹⁶⁶ Because furosemide results in a pronounced loss of water, Na⁺, and titratable acid from (primarily) the extracellular fluid compartment, this results in increased plasma [Na⁺] (by 8 mEq/L)

and decreased plasma [Cl⁻] (by 8 mEq/L) between 5 and 10 h after intramuscular administration of 1 mg/kg.¹⁶⁷ In this study, it appears that plasma [SID] increased by 13 mEq/L 8 h after administration. More recently, a 7 mEq/L increase in plasma [SID] 4 h after administering 1 mg/kg furosemide has been reported.¹⁶⁸ This large increase in [SID] fully explains the increase in pH (7.383 to 7.445) and increase in [HCO₃⁻] (3 mEq/L) in arterial plasma.¹⁶⁸ The effect of increased [SID] alone would have been greater; however, the concomitant increase in [PP] and hence [A_{tot}], due to the extracellular dehydration, results in an acidifying effect. Arterial and mixed venous PCO₂ remains unchanged in resting horses.¹⁶⁸ During high-intensity treadmill running exercise (4 h after 0.5 or 1 mg/kg furosemide administration), the elevations in arterial and mixed venous pH and [HCO₃⁻] were maintained in furosemide-treated horses through to the last minute of maximal exercise; furosemide did not appear to result in increased lactate accumulation within blood.¹⁶⁸ Similar responses were reported by Hinchcliff & McKeever.¹⁶⁹

In many ways, the alkalosis resulting from furosemide administration qualitatively resembles that resulting from administration of NaHCO₃ and related alkalizing agents. The main feature in common that produces the increased plasma pH and [HCO₃⁻] is the pronounced decrease in plasma [Cl⁻]. The main differences are elevations in [PP] and [Na⁺] with furosemide, while these variables remain largely unchanged with alkalizing agents. The alkalosis resulting from even high-dose furosemide (1 mg/kg) is relatively mild compared to the moderate alkalosis resulting from high-dose NaHCO₃ (>0.5 mg/kg), although the effects appear to be similar in duration.

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Thermoregulation and exercise-associated heat stress

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Body heat is produced by metabolism and is also gained from the environment. In homeotherms, internal temperature is normally maintained within a narrow range (37–40°C) by integrated neurophysiologic mechanisms that balance heat production and heat loss. Thermoregulation is the process by which the internal temperature is regulated to maintain body temperature within this thermoneutral zone. For example, during heat exposure or exercise, when heat gain occurs, the thermoregulatory system will provoke mechanisms for heat loss such that the rise in internal (or core) body temperature is mitigated. It is the most important regulation system in homeothermic animals.¹

In horses, as in humans and other mammalian species, an excessive elevation in body temperature limits performance capacity. Therefore, a thorough understanding of thermoregulation and means for enhancement of these mechanisms is crucial to management of the athletic horse. The fact that horses often train and compete in hot weather, ambient conditions that substantially increase the risk of thermal injury, further underscores the importance of such understanding.

Heat production and dissipation

Core body temperature is a dynamic equilibrium between factors that add or remove heat. This balance is maintained by integration of mechanisms that vary the body's rate of heat production, alter the transfer of heat to the periphery (e.g. skin), and regulate evaporative cooling. The hypothalamus contains the central coordinating center for the various processes of thermoregulation. Specialized neurons within the hypothalamus act as a thermostat that initiates ther-

moregulatory adjustments to deviations from normal body temperature. Heat-regulating mechanisms are activated by thermal receptors in the skin that provide input to the hypothalamus, or by direct stimulation of the anterior hypothalamus via changes in the temperature of blood perfusing the area.

The greatest disequilibrium in heat balance occurs during exercise. Conversion of chemical energy (i.e. stored substrates) to mechanical energy (e.g. muscular contraction) is inefficient, with approximately 75–80% of the total chemical energy released as heat rather than physical work.² As such, the rate of metabolic heat production increases markedly with the onset of exercise and is accompanied by increases in muscle and core body temperatures. The increase in core temperature provokes activation of heat dissipatory mechanisms such that increases in body temperature are mitigated. However, whether balance between heat gain and heat loss can be re-established will depend on the duration and intensity of exercise and the efficiency of heat dissipation. The latter is primarily influenced by ambient conditions but also modified by physiological adaptations (e.g. conditioning, heat acclimatization) in heat dissipatory mechanisms.

Heat production

For living organisms, biologic work is either external, which includes moving the body or other objects through muscular contraction, or internal work that would include all other forms of biologic work, such as smooth muscle contraction, synthesis of molecules and compounds, or active transport within cells. With the exception of periods of growth, ultimately all this work is transformed into heat that is either stored or liberated. Energy expenditure

by the body can therefore be expressed by the following equation:

$$\text{Total energy expenditure} = \text{internal heat production} + \text{external work performed} + \text{energy stored}$$

The body has a basal level of energy expenditure or basal metabolic rate (BMR) that is lowest when environmental temperature range is within a thermoneutral zone. This BMR minimizes energy expenditure required to maintain normal body temperature. The BMR is altered by numerous factors, both internal and within the environment, which increase or decrease energy expenditure. Metabolic rate is normally lowered during sleep and increases with any form of work or stress. Perturbations of BMR, such as exercise, fever, catecholamine release, feed consumption (thermic effect of feed), or dealing with a cold environment, can therefore affect heat balance. In mammals, there have been extensive investigations of the relationship between bodyweight and heat production,^{2,3} which have demonstrated that resting heat production is proportional to body mass to the power of 0.75 in adults but not in growing animals. There are, however, differences between species and it could be expected that within a species such as the horse with extensive variation in size and weight based on breed, lighter breeds may have a significantly lower resting heat production when compared to heavier breeds.

Heat production in horses is influenced by dietary factors, including the quantity and quality of feed and water intake. Replacing hay in the diet with grains has been demonstrated to decrease heat production,⁴ with a further reduction in heat production possible with fat supplementation.⁵ When exposed to hot or cold environments, water restriction and dehydration can also reduce heat production.

During exercise, the workload or speed is the main determinant of the rate of heat production. Other factors such as the weight of rider and tack, and the nature of the terrain and footing will also contribute to the overall workload.^{6,7} Heat production during exercise can be estimated from oxygen consumption data:

$$\text{Metabolic heat} = \dot{V}O_2 \text{ (liters per min)} \times k \times \text{exercise duration (min)}$$

where $\dot{V}O_2$ = oxygen consumption and k = amount of heat liberated per liter of oxygen consumed. Values for k range from 4.7 kcal to 5.1 kcal depending on the substrate oxidized (lowest value for pure fat oxidation, highest value for pure carbohydrate oxidation).³

Metabolic rate in horses is 40- to 60-fold higher during exercise at maximum oxygen uptake ($\dot{V}O_{2 \text{ max}}$) when compared to the resting state. For a 500 kg horse with a $\dot{V}O_{2 \text{ max}}$ of 80 L/min, this equates to metabolic heat production in excess of 400 kcal/min (~1.3 MJ/min) of exercise. Production of this quantity of heat without any ability for heat dissipation would result in an increase in body temperature of approximately 1°C per minute during exercise. Although the rate of metabolic heat production is lower during endurance exercise, the overall heat load is substantially higher because of the longer work duration. For example, it has been estimated that the metabolic heat production of an endurance horse running at 8 m/s is about 150–200 kcal/min; if no heat were dissipated, this heat load would result in an increase in core temperature of approximately 21°C per hour. These hypothetical measurements emphasize that effective heat loss mechanisms are crucial and serve to further underline the additional impact that severe ambient conditions will impose on the horse's ability to lose heat to the surrounding environment.

In contrast to most other large domestic species in which skeletal muscle comprises 30–40% of total bodyweight, half the total bodyweight of the Thoroughbred is working muscle. This higher percentage of bodyweight from muscle contributes to the horse's higher mass-specific $\dot{V}O_2$ when compared to other athletic species, including humans. Furthermore, a running horse uses a greater proportion of its body mass for locomotion than does a human performing running or leg cycling exercise. Thus, the mass-specific heat load for the exercising horse is as much as 2- to 3-fold higher compared to that of exercising humans. Despite a substantially higher rate of heat production in the horse, the ratio of surface area to body mass is approximately 50% less than that of humans (man = 1:35–40 m²/kg; horse = 1:90–100 m²/kg).^{6,7} As a result, the horse has a significantly smaller surface area over which to dissipate a relatively larger metabolic heat load and, at any given workload, must dissipate approximately four times more heat per unit of body surface area during exercise than human athletes. The disadvantage posed by a smaller surface area:body mass ratio can be partially offset by higher rates of cutaneous and respiratory heat loss. However, it is apparent that exercise is a considerable thermoregulatory challenge to the horse, with prolonged exercise representing one of the most demanding situations.

Mechanisms of heat transfer

Conductive, convective, radiative, and evaporative heat loss are the four basic mechanisms for heat transfer.

Heat loss by *conduction* involves direct transfer of heat through a liquid, solid, or gas from one molecule to another. Although most of the body heat is transferred to the periphery by the circulation, a small amount moves by conduction directly through the deep tissues to the cooler surface. Heat loss by conduction then occurs by the warming of air molecules and cooler surfaces in contact with the skin. The rate of conductive heat loss is directly proportional to the temperature gradient between the skin and surrounding surfaces, and inversely proportional to the thickness of the hair coat. Heat loss by conduction from the surfaces of the head, neck, and distal limbs is more effective due to a higher surface area to mass ratio in these regions when compared to proximal limbs, thorax, and abdomen.

Convection represents the transfer of heat between two media, such as the skin surface and surrounding air. The effectiveness of heat loss by convection depends on how rapidly the air near the body is exchanged once it is warmed. Conductive heat loss is most effective when the warm air surrounding the body is continually replaced by cooler air, as occurs as a running horse moves through the air and/or wind speed is moderate to high. On the other hand, the trapping of air within a long hair coat will impede convective heat transfer to the environment. Convective heat transfer also occurs in the respiratory tract, the rate of which is dependent on pulmonary ventilation and the temperature difference between inspired and expired air.

Radiative heat transfer occurs when electromagnetic radiation is emitted or absorbed at the skin surface. As body temperature is normally higher than the environment, there is a net loss of radiative heat energy at the skin surface. However, in hot ambient conditions, when the temperature of objects in the environment exceeds skin temperature, radiant heat energy is absorbed from the surroundings. Under these conditions, the only avenue for heat loss is evaporative cooling. A gain of radiant heat energy also occurs via direct (or reflected) sunlight. It has been suggested that solar radiation can contribute up to 15% of the heat gain in horses during exercise in sunny conditions.⁸

For the horse, the most important mechanism for heat loss is *evaporative* cooling including the evapora-

tion of sweat from skin surfaces and water from the respiratory tract. The efficacy of this mechanism is dependent upon the extent of the vapor pressure gradient between body surface and environment. Calculations of the estimated heat loss are based on the latent heat of vaporization of water (from a liquid to a vapor — 598 kcal [2501 kJ] for each gram of water at 0°C).⁹ After accounting for possible variations in the thermodynamic properties of sweat when compared to water, it is estimated that the evaporation of 1 L of sweat from the skin surface will dissipate approximately 580 cal (2428 kJ or 2.4 MJ) of body heat.¹⁰ The quantity of heat (~2.4 MJ) dissipated in association with the evaporation of 1 L of sweat in thermoneutral conditions is approximately equivalent to the heat generated by 2 min of high-intensity exercise or 6 min of moderate-intensity exercise. In optimum conditions (i.e. when relative humidity is low), the evaporation of sweat is a very efficient mechanism of heat loss and can account for as much as 65% of total heat loss during exercise. However, several environmental factors will influence the efficacy of evaporative heat loss. These include ambient temperature and relative humidity, the extent of the vapor pressure gradient between the skin surface, and the rate of air movement.^{11–13} At high ambient humidity, the vapor pressure gradient between the body surface and the environment narrows thereby constraining evaporative cooling and increasing the rate of heat storage.

The extensive surface area of the respiratory tract also provides a mechanism for heat dissipation. This process relies upon the difference in vapor pressure between the inspired air and that of the epithelial surface of the respiratory tract. The external nares of the horse contribute considerable surface area for heat exchange. Similarly, the extensive surface area of the upper respiratory tract, including the internal nares and nasal turbinates, provides an environment in which air entering the nasal passages contacts the highly vascularized epithelium of the upper respiratory tract. Horses also have a unique anatomical arrangement by which their internal carotid arteries are enveloped by a pair of air-filled guttural pouches. Preliminary studies suggest that exercising horses can use their guttural pouches to cool blood en route to the brain.¹⁴ It is surmised that this heat loss from the upper respiratory tract contributes to selective brain cooling, whereby the temperature of blood reaching the brain is lower when compared with that measured in mixed venous (pulmonary artery) blood or within skeletal muscle.¹⁵ Heat loss from the respiratory tract is dependent upon relative humidity and minute ven-

tilation. Under cool, dry conditions, the extent of heat loss via this mechanism is estimated to be between 15 and 25% of total heat loss. In hot, humid conditions, when cutaneous evaporative cooling is compromised, respiratory heat loss may account for a relatively higher proportion of total heat loss and therefore represent 25% or more of total heat loss. While elevations in the respiratory rate increase the proportion of heat loss from the respiratory tract in these conditions, mechanical limitations imposed during exercise (e.g. the coupling of stride to respiration during canter and gallop) may ultimately limit heat loss from the respiratory tract.

Mechanisms of sweat formation

In only a limited number of species, including some bovidae, primates, and equidae, is the sweat gland primarily a thermoregulatory organ.^{4,16} In the horse, sweat glands are present in both haired and relatively hairless skin with regional variation in the density of glands that is not dependent on the presence of a hair coat. Structurally, the gland is similar to that of many other domestic species, consisting of a fundus and a duct connecting the fundus with the skin surface. Throughout most of its length, the duct lining is composed of two layers, with a single layer of keratinocytes lining the duct at the skin surface. Located within the dermis, the fundus is lined by an inner layer of secretory epithelium interspersed with myoepithelial cells and surrounded by a fenestrated sheath of fibrocytes that encloses a layer of connective tissue. Although sweating appears to be under sympathetic nervous control, there is no evidence of direct sympathetic innervation of the sweat gland. Rather, sweating appears to occur via humoral stimulation of

β_2 -adrenergic receptors on sweat glands. As a result, sweating can be initiated by epinephrine (adrenaline) release in advance of any stimulus related to an increase in core temperature.

As most studies to date have investigated sweating rate and composition in Thoroughbred horses, the degree to which sweating rate and composition vary between breeds is unknown. To date, it appears that the basic composition of sweat is similar between breeds. Equine sweat, unlike that of humans, is isotonic to slightly hypertonic relative to plasma.^{11,15,17} Sweat ion concentrations are largely a reflection of sweating rate and therefore are subject to alteration based on environmental conditions and exercise intensity (Table 6.3.1). Although there is some variation in sweat ion composition of equine sweat, individual differences in sweat composition do not appear to be as extensive in horses when compared to human athletes. Epinephrine (adrenaline) infusion will produce a more dilute sweat and may account for the less concentrated sweat produced during high-intensity exercise when compared to low-intensity exercise.^{11,15,18}

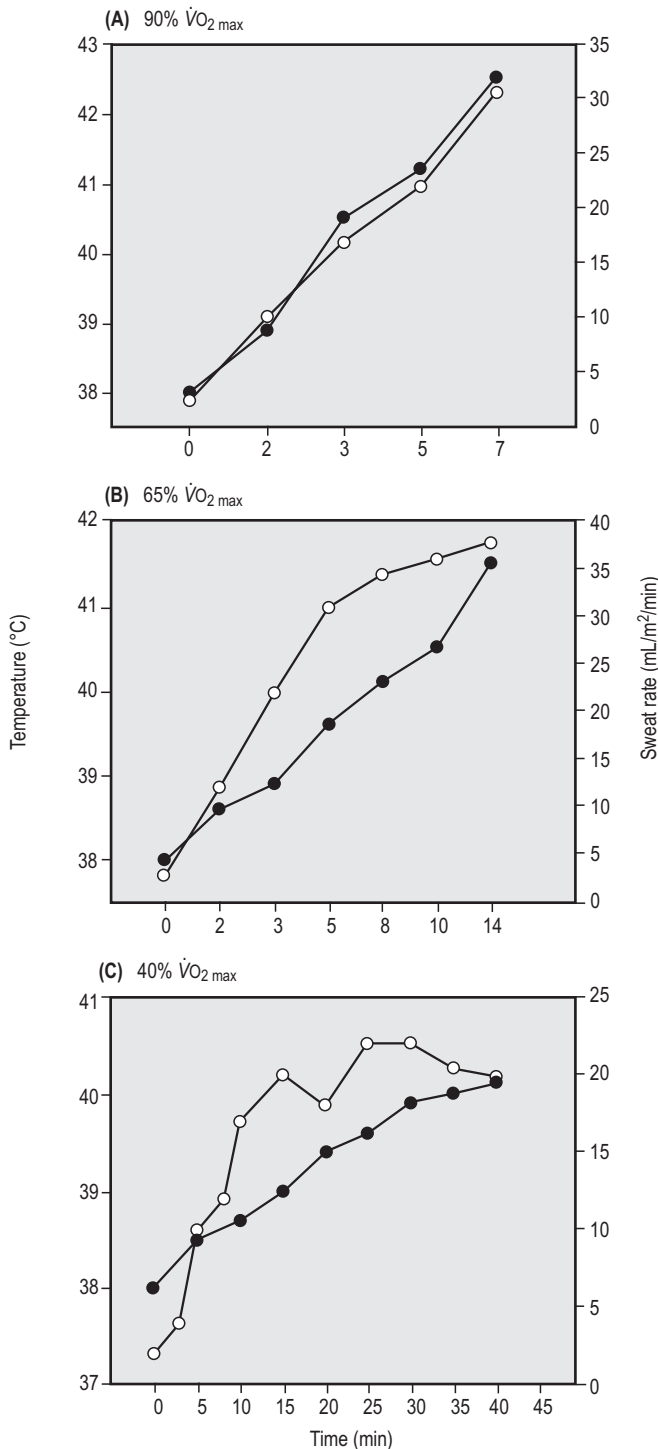
During exercise, an increase in body temperature as a result of metabolic heat production is the primary stimulus for sweating. Normally, sweating is initiated at a specific core temperature and continues in proportion to the increase in core temperature. Hodgson et al¹⁹ demonstrated that increases in sweating rate at three different exercise intensities (40%, 65%, and 90% of $\dot{V}O_{2\text{ max}}$) were closely related to elevations in carotid artery blood temperature (Fig. 6.3.1). Rate of rise in body temperature and the concentration of circulating catecholamines associated with different exercise intensities could also contribute to the determination of sweating rate.

Table 6.3.1 Sweating rate, ion concentrations, and osmolality in horses at two exercise intensities and three different ambient conditions after 10 min exercise

Variable	CD high	CD low	HD low	HH low
Sodium (mmol/L)	124.0 $\pm$ 6.7	116.7 $\pm$ 6.1	133.6 $\pm$ 2.3	130.6 $\pm$ 1.7
Potassium (mmol/L)	25.8 $\pm$ 2.1	32.6 $\pm$ 1.4	41.5 $\pm$ 1.1	28.1 $\pm$ 0.9
Chloride (mmol/L)	142.0 $\pm$ 5.6	144.3 $\pm$ 4.3	155.8 $\pm$ 3.2	149.5 $\pm$ 2.9
Osmolality (mOsm/kg)	313 $\pm$ 18	303 $\pm$ 6	339 $\pm$ 6	327 $\pm$ 5
Sweating rate (mL/m ² /min)	40.4 $\pm$ 3.7	21.1 $\pm$ 5.2	32.8 $\pm$ 5.1	27.0 $\pm$ 6.2

CD, cool, dry (room temperature [T] = 20°C, relative humidity [RH] = 45–55%); HD, hot, dry (T = 32–34°C, RH = 45–55%); HH, hot, humid (T = 32–34°C, RH = 80–85%); high, exercise at 90% of maximum oxygen consumption ($\dot{V}O_{2\text{ max}}$); low, exercise at 50% of $\dot{V}O_{2\text{ max}}$.

Data from references^{11,82}.

**Fig. 6.3.1**

Carotid artery temperature (closed circles) and sweat rate (open circles) in horses exercising at 90% (A), 65% (B), and 40% (C) of maximal $\dot{V}O_2$ uptake ($\dot{V}O_{2\text{ max}}$). Ambient temperature was 21–23.5°C. (Adapted from Hodgson et al.¹⁹)

Thermoregulation during exercise

During exercise, metabolic heat from working muscles must be transferred to the skin surface to be lost to the environment. Peripheral thermoreceptors in skin, spinal cord, skeletal muscle, abdomen, and hypothalamus detect changes in thermal load and produce a proportional output that is integrated in the hypothalamus to allow adequate thermoregulatory effector activity, particularly by the circulatory system and sweat glands. The primary physiologic mechanisms driving heat loss are an increase in the proportion of cardiac output directed toward the cutaneous circulation and an increase in the rate of sweat secretion. The increase in cardiac output and blood flow to contracting muscles enables a substantial increase in convective heat transfer away from the muscle. The circulation carries the heat to the body core, resulting in an increase in core temperature. Increasing core temperature and, to a lesser extent, increasing skin temperature provide the afferent signal for reflex increases in skin blood flow and sweating, thereby facilitating heat transfer to the skin surface and its dissipation to the environment.

Skin blood flow is substantially increased by the opening of capillary beds that are normally bypassed by arteriovenous anastomoses that connect arteries directly to veins. The increase in blood flow through the vascular beds of the skin allows heat to be lost to the environment via convection and direct radiation of heat from the skin surface. The efficacy of transfer of heat by convection and radiation varies according to the rate of air movement across the skin (wind speed) and the gradient of skin temperature to environmental temperature. Increased skin blood flow also provides the latent heat for vaporization of sweat and as well as the fluid required for sweat production. The attempt to maximize blood flow for increased activity and thermoregulation is also reflected in decreased splanchnic and adipose tissue blood flow.²⁰ Greater oxygen demand increases respiratory rate and respiratory blood flow and both activities will enhance the extent of evaporative cooling by the respiratory system.

Sweating rates of ~20–55 mL/m²/min have been measured on the necks and backs of horses exercising on a treadmill in a laboratory.^{19,21–23} Assuming a body surface area of 4.5–5.0 m² for a 500 kg horse, these sweating rates correspond to fluid losses of 6–15 L per hour. This estimate of hourly sweat fluid loss is in

agreement with sweat rates calculated on the basis of the decrease in body mass during prolonged exercise under field conditions.²⁴ When expressed in terms of sweating rate per unit area of skin, these sweating rates are 2- to 3-fold greater than those reported for human subjects.

At any given point in time during exercise, core body temperature reflects the balance between heat production and dissipation. Soon after the onset of exercise, the rate of heat production greatly exceeds the rate of heat dissipation such that there is a rapid increase in muscle temperature.²⁵ During short-term, high-intensity exercise (e.g. racing), the rate of heat production will exceed the rate of heat loss throughout exercise and body temperature will continue to increase until the cessation of exercise. In this circumstance, a large proportion of the metabolic heat load will be dissipated during the recovery period. Conversely, during more prolonged low- to moderate-intensity exercise in temperate ambient conditions, activation of heat dissipatory mechanisms progressively attenuates the rate of rise of body temperature. Eventually, the rate of heat loss increases sufficiently to balance metabolic heat production, allowing a near steady-state core temperature to be achieved.²⁵

Effects of environmental heat load on exercise responses

Not surprisingly, the thermal response to exercise is affected by the ambient conditions. As environmental temperature increases, the thermal gradient between the skin and the environment is reduced, and sensible heat loss (i.e. convective and radiative heat transfer) is impaired. When ambient temperature exceeds skin temperature (>35–36°C), the gradient for heat transfer is reversed and the body gains heat from the environment. If humidity is low, a decrease in sensible heat loss can be offset by an increase in sweating rate and evaporative cooling. As humidity rises, the gradient between skin and ambient dew point is reduced and evaporative heat loss is also impaired. The decrease in sweat evaporation is manifested by excessive wetting of the skin surface and drippage of sweat from the body. Sweat that drips from the body only removes 5–10% of the heat that can be dissipated by evaporation of sweat. Therefore, during exercise under conditions of high ambient heat and humidity, the rate of heat dissipation may be inadequate to prevent the progressive rise in body temperature. The impact of the environment on the rate of rise of core body

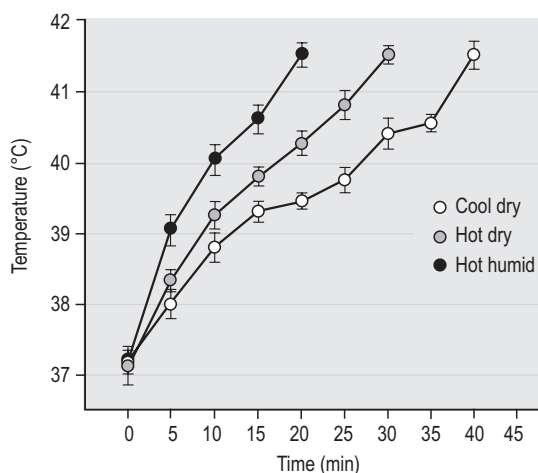


Fig. 6.3.2

Time course of rise in pulmonary artery blood temperature (T_{pa}) during exercise at 50% of maximal O_2 uptake in cool, dry (room temperature [T] = 20°C; relative humidity [RH] = 45–55%), hot, dry (T = 32–34°C, RH = 45–55%), and hot, humid (T = 32–34°C, RH = 80–85%) ambient conditions. Exercise was discontinued when T_{pa} reached 41.5°C. (Adapted from Geor et al.¹³)

temperature in exercising horses is depicted in Figure 6.3.2. The rate of heat storage when exercising in hot, humid conditions may be more than twice the rate occurring during exercise at the same intensity in cool, dry conditions.^{13,26–28}

Increased demands for respiratory heat loss are reflected by an increase in respiratory rate during and after exercise. Kohn & Hinchcliff²⁹ reported a 20–25% increase in the respiratory rate of horses during speed and endurance tests in hot when compared to cool conditions. In laboratory experiments, an approximately twofold increase in post-exercise respiratory rate has been observed in horses under hot, humid when compared to cool, dry environmental conditions.^{13,26} When ponies were exposed to heat (41°C dry bulb temperature), there were threefold increases in respiratory rate and blood flow to tissues of the upper respiratory tract,³⁰ reflecting the role of the respiratory system in heat dissipation. Similarly, during moderate-intensity (~30% $\dot{V}O_{2\max}$) exercise respiratory rate was fivefold greater in hot than in thermoneutral conditions.²⁰

An important consequence of the impairment to heat dissipation during exercise in the heat is a decrease in the time to attainment of a critical upper limit in core body temperature. In humans, it is clearly established that time to exhaustion in trained subjects

during exercise in the heat is inversely related to the initial level of body temperature and directly related to the rate of heat storage.³¹ That the lowering of core temperature prior to the start of exercise³² or cooling the body during the period of exercise³³ will delay attainment of the critically high body temperature and extend exercise duration is further evidence for the relationship between body temperature and exercise performance.

Several factors may contribute to a decrease in performance when exercise is undertaken in hot versus cool conditions. These include the effects of hyperthermia on brain and muscle function, and compromise of cardiovascular and respiratory function. There appears to be a critical body temperature above which mammals will not continue to exercise voluntarily, likely a protective mechanism to protect the human or animal from reaching tissue temperatures that harm cell function.²⁸ Thus, a more rapid attainment in critical body temperature will translate to a reduction in exercise duration. In trained humans exercising over a range of work intensities in the heat, voluntary fatigue occurs at a core (esophageal) temperature of 39.7–40.0°C.^{31,34–36} Furthermore, following heat acclimation procedures that enhance thermoregulatory mechanisms and reduce the rate of heat storage during exercise, the core temperature at the onset of fatigue is unchanged.^{35,37} Measurements of central blood (pulmonary artery) temperature in horses during heavy exercise have demonstrated that fatigue occurs as blood temperature approaches 42.5–43°C;^{19,38} muscle temperature may reach 44–45°C during such high-intensity exercise. Hypothalamic blood temperature, on the other hand, is approximately 1°C lower than central blood temperature in horses during heavy exercise in moderate ambient conditions.¹⁵ The difference between the temperatures in these two regions provides evidence for the existence of a mechanism for selective brain cooling in the horse. Particularly during exercise in the heat, the onset of fatigue at some critical upper limit in brain temperature may represent a mechanism to avoid heat stroke.

Human studies, dating back to the work of Asmussen and Boje,³⁹ have indicated that a moderately elevated, but steady-state core body temperature is advantageous to muscle function and to the dissociation of oxygen from red blood cells within muscle tissue. The increase in muscle temperature acts on glycolytic and glycogenolytic enzymes, altering flux rate through these pathways.^{40,41} This Q_{10} effect is accentuated during exercise in the heat. However, at

high muscle temperature (>46°C) deleterious structural and functional alterations in skeletal muscle proteins can be induced.⁴² These proteins play essential roles in mitochondrial respiration, regulation of calcium by the sarcoplasmic reticulum and the subsequent interactions of myosin and actin, and control of electrolyte movement across the sarcolemma.^{31,43} As a consequence, substantial detrimental alterations to skeletal muscle metabolism may occur with elevation of muscle temperature to this critical range.

During exercise heat stress, circulatory adjustments must be regulated to maintain adequate blood flow to contracting muscle and to the thermoregulatory tissues, particularly the skin and the upper respiratory tract.¹⁰ Given a finite cardiac output, the increased demands for blood flow to these thermoregulatory tissues may compromise blood flow to skeletal muscle, thereby limiting oxygen delivery and, possibly, exercise duration. Recent studies in ponies have provided data on the effects of environmental heat load (41°C dry bulb) on the redistribution of cardiac output during exercise.²⁰ Blood flow to the fore- and hindlimbs during moderate- (~30% $\dot{V}O_{2\max}$) and high-intensity (~65% $\dot{V}O_{2\max}$) exercise was reduced by as much as 25–30% when compared to similar exercise in thermoneutral conditions. This reduction in muscle blood flow during exercise heat stress is likely to restrict performance and contribute to an early onset of fatigue.

A reduction in maximal aerobic power is another potential reason for decreased physical performance during exercise in the heat. A reduction in peak oxygen uptake has been demonstrated in human subjects exercising in the heat.^{44–46} When non-heat-acclimatized horses performed an incremental treadmill exercise test in hot, humid conditions (temperature 30°C, relative humidity 75%), peak expired minute ventilation, oxygen uptake, and oxygen pulse were significantly lower and plasma lactate concentrations higher when compared to exercise in temperate conditions (15°C, 55% relative humidity).⁴⁷ Although the mechanism of the reduction in peak oxygen uptake in horses exercising in heat and humidity has not been determined, it is possible that alterations in breathing strategy that favor heat loss from the upper respiratory tract (dead space ventilation) and compromise of skeletal muscle blood flow result in decreased oxygen uptake and delivery. Regardless of mechanism, such a reduction in peak oxygen uptake is likely to impair performance relative to exercise performed in temperate conditions.

Physiologic factors affecting thermoregulatory capacity

The term 'exercise heat tolerance' refers to an ability to withstand high internal and external heat loads during exercise. In humans it is well recognized that high aerobic fitness and a period of acclimatization in the heat improves both physiological and psychological responses to the challenge of exercise in the heat.⁴⁸ Adaptations in heat dissipatory mechanisms improve cardiovascular stability, decrease the rate of heat storage, and increase the duration of exercise before volitional fatigue. However, highly trained athletes are also able to tolerate higher levels of hyperthermia when compared to untrained individuals; that is, volitional fatigue occurs at a higher core temperature.⁴⁹ Conversely, regardless of training or heat acclimatization, dehydration decreases exercise heat tolerance, as evidenced by an increase in the rate of heat storage and the development of volitional fatigue at a lower level of hyperthermia. There is now evidence that these physiological factors also modify the thermoregulatory capacity of horses. Specifically, conditioning and heat acclimatization improve tolerance to exercise in the heat, whereas dehydration adversely affects heat dissipation in horses during exercise.

Other factors that can influence thermoregulation in exercising horses include coat color and the density of the hair coat. Coat color will affect the quantity of solar heat absorbed, while a long hair coat will limit evaporative heat loss.

Conditioning

Physical training in a cool environment is broadly accepted to improve exercise heat tolerance. The extent to which heat dissipatory effector mechanisms are stimulated and the duration of that stimulus will determine the effectiveness of training in improving exercise heat tolerance. Detectable changes in heat tolerance are evident in human athletes after 1–2 weeks of training but are much more substantial if regular training is sustained for 8–12 weeks.^{50–53} In human subjects, the stimulus must be sufficient to elevate core temperature by approximately 1.5–2.0°C for a minimum of 30 min/day.^{50,52,53}

At a given percent of maximal oxygen uptake ($\dot{V}O_{2\max}$), trained athletes have a higher metabolic rate and, hence, greater heat production at any given relative exercise intensity. Despite this higher metabolic

rate, the trained individual is able to maintain a similar core temperature when compared to the untrained subject indicating an enhanced ability to dissipate heat. Improved cutaneous blood flow and whole-body sweating have been measured in trained individuals and are the main contributions to more effective heat dissipation in human subjects.⁵⁴ However, highly trained human athletes have been shown to have less extensive sweat fluid losses during exercise^{55,56} and there is evidence for similar sweating economy in horses following training.⁵⁷ Specifically, 8 weeks of moderate-intensity treadmill conditioning resulted in a 1.6-fold increase in sweating sensitivity and an approximately 0.7°C decrease in sweating threshold in horses during exercise in hot, dry conditions (temperature 32–24°C, relative humidity 45–55%). Despite higher sweating rates for a given core temperature during exercise, decreases in recovery sweating rates resulted in an overall reduction in sweat fluid losses (Fig. 6.3.3).⁵⁷ This enhancement in sweating economy as a result of training may assist in minimizing demands placed on the circulatory system by reducing the extent of dehydration associated with fluid losses. Alterations in sweat composition following training in horses appear to reflect changes in the rate of sweat production rather than a modification of the sweat gland itself.^{23,57}

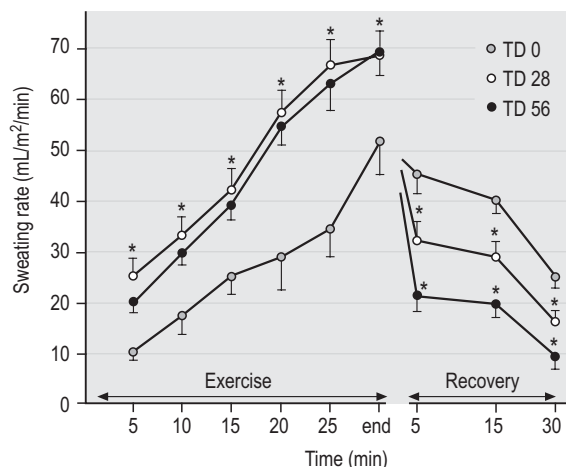


Fig. 6.3.3

Mean sweating rate for five horses averaged for 5-min intervals during an exercise test in hot, dry conditions (32–34°C and 45–55% relative humidity) at 50% of maximal $\dot{V}O_{2\max}$ on training days (TD) 0, 28, and 56 of an 8-week training regimen. *Significantly different from TD 0 ($P < 0.05$). (Data from McCutcheon & Geor.⁵⁷)

Improvements to cardiovascular function are one of the hallmarks of training. The increases in plasma volume, stroke volume, and cardiac output all contribute to increased cardiac stability during exercise and, in particular, a lower heart rate at the same work output.⁵⁸ Expanded plasma volume in the trained athlete also improves thermoregulatory capacity by reducing the extent to which cutaneous blood flow is compromised. Increased resting plasma volume reduces the risk of a fall in cardiac output during exercise in the face of the decline in plasma volume associated with sweat fluid losses. In studies of human subjects, Roberts et al⁵⁹ observed earlier onset of cutaneous vasodilation in trained individuals. Similarly, in more recent work, Fritzsche & Coyle⁵⁴ demonstrated that cutaneous blood flow was higher in trained than in untrained individuals at the same relative workload despite similar core (esophageal) body temperatures. In the horse, as in human subjects, enhanced capacity for heat dissipation is, in part, a reflection of an expansion of blood volume⁶⁰ and likely a higher proportion of blood flow directed to the skin surface.

The time course of decay in training-induced thermoregulatory adaptations after the onset of detraining has not been determined in horses. In humans there is a strong association between aerobic fitness and exercise heat tolerance, and the decline in thermoregulatory function with detraining tends to parallel the reduction in aerobic fitness.^{49,50}

Heat acclimatization

The term 'acclimatization' is used to describe adaptive changes that occur when a subject undertakes repeated, prolonged exposure to severe environmental conditions (*acclimation* refers to the adaptations produced in controlled laboratory conditions). In the context of the present discussion, hot conditions are normally considered those that will induce the greatest thermoregulatory challenge for horses. As indicated previously, exercise training in a cool environment improves physiological responses when horses exercise in hot conditions. The adaptive responses to training and acclimatization are qualitatively the same. However, the more profound thermal stimulus used during acclimatization will result in thermoregulatory adaptations of a greater magnitude.^{60,61} Furthermore, factors that modify the environment in which acclimatization is undertaken will influence the extent of alterations. These differences are apparent in the higher rate of sweat production

during exercise when acclimatization occurs in hot, dry versus hot, humid conditions.⁶²

Human studies have demonstrated that the process of heat acclimation begins within a few (3–5) days of regular exposure to and exercise in the heat, with most adaptations complete within a 14-day period.^{35,37,50,52,61} The most notable changes are an increase in plasma volume, a decrease in heart rate and core temperature during exercise, an increase in sweating rate and initiation of sweating at a lower body temperature, and redistribution of cardiac output such that there is an increase in blood flow to capillary beds of the skin. In general, the cardiovascular adaptations are complete during the first week of acclimation, whereas alterations in sweating responses require 10–14 days of repeated heat exposure.^{60,61} Heat acclimation also may result in an improved efficiency of biochemical energy transformation in contracting muscles, thereby attenuating heat production in the acclimated versus unacclimated state.²⁶

In horses, there is evidence that regular exercise in the heat results in physiological adaptations that are consistent with thermal acclimation. Marlin and co-workers³⁸ subjected five horses to 15 consecutive days of treadmill exercise at 30°C and 85% relative humidity. Training consisted of a combination of low-, medium- and high-intensity exercise, with a total duration of exercise heat exposure of 80 min per day. Before and after acclimation, horses undertook a treadmill exercise test designed to simulate the speed and endurance test of a three-day event at 30°C and 85% relative humidity. Following acclimation, four of the five horses were able to complete a significantly greater amount of phase D in the exercise test (pre: 6.3 ± 0.3 min; post: 7.3 ± 0.3 min; target time = 8 min), suggesting an improvement in heat tolerance. Resting body temperatures (rectal, pulmonary artery, tail skin) were lower after acclimation, whereas plasma volume was unchanged.³⁸

In another study, six conditioned horses were exposed for 4 h to heat and humidity (temperature 33–35°C, relative humidity 80–85%) for 21 days.^{63–66} One hour of low- and moderate-intensity treadmill exercise (30–60% $\dot{V}O_{2\max}$) was completed during daily heat exposure. At regular intervals during the 21-day period, horses undertook a standardized submaximal (50% $\dot{V}O_{2\max}$) exercise test in the same environmental conditions. In addition, before and after 18 days of acclimation, the horses performed the same exercise test in hot, dry conditions (relative humidity 45–50%). Exercise duration was defined as the time taken for attainment of a pulmonary artery blood temperature

Table 6.3.2 Run time during treadmill exercise in hot and humid conditions, pre-exercise body mass and change in body mass on days 0, 3, 7, 14, and 21 of heat acclimation in trained Thoroughbred horses

	Run time (min)	Body mass (kg)	Total sweat fluid loss associated with SET (L)	Sweat loss during exercise (mL)	% of total loss (exercise)	Sweat loss during recovery (mL)	% of total loss (recovery)
Day 0	19.09 ± 1.41	454.1 ± 11.7	11.67 ± 1.23	3844 ± 380	33.8 ± 2	7413 ± 953	66.2 ± 2
Day 3	20.92 ± 1.98	452.0 ± 12.0	12.00 ± 0.86	4179 ± 645	38.0 ± 4	6452 ± 818	62.1 ± 4
Day 7	19.59 ± 1.70	449.5 ± 11.5	11.50 ± 0.99	4324 ± 756	39.8 ± 3	5772 ± 875	59.8 ± 3
Day 14	20.42 ± 1.78	449.7 ± 10.1	10.08 ± 1.31	4451 ± 577	41.7 ± 3*	5323 ± 444	58.0 ± 3*
Day 21	19.61 ± 1.86	445.7 ± 8.7	8.67 ± 0.71*	4452 ± 490	50.7 ± 3*	4193 ± 435*	49.1 ± 3*

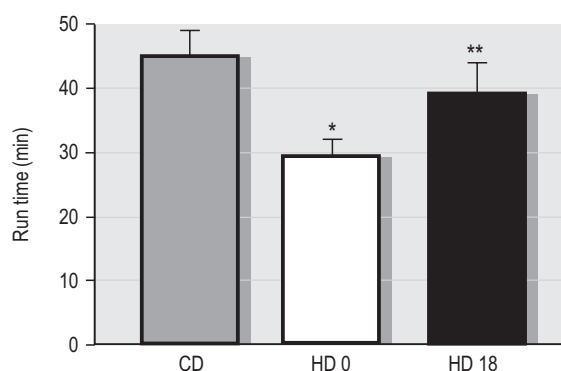
Values are mean ± SE for six horses. Run time represents duration of exercise at 50% $\dot{V}O_{2\text{ max}}$ to attainment of a pulmonary artery temperature of 41.5°C. Total sweat fluid loss includes exercise and 1 h of recovery. Exercise includes exercise at 50% $\dot{V}O_{2\text{ max}}$ only. Recovery includes 1 h of recovery only.

SET, standardized exercise test.

*Significantly different from day 0, $P < 0.05$.

of 41.5°C. Similar to the findings of Marlin et al.³⁸ there were significant decreases in resting body temperatures after 5–7 days of heat acclimation. However, in contrast to Marlin's findings, there was a 7–10% increase in plasma volume during the first week of heat acclimation. Thereafter, plasma volume tended to decrease and was not different from pre-acclimation values at the end of the experimental period.⁶⁵ There was a 25% increase in exercise duration when comparing trials performed in the hot, dry conditions before and after acclimation (Fig. 6.3.4). However, heat acclimation did not increase exercise duration in the hot, humid environment,⁶⁴ further emphasizing the biophysical limitations to heat dissipation in such conditions ('uncompensable heat stress').

Sweating rates greatly exceeded evaporative capacity throughout the 21-day period of humid heat exposure, as evidenced by profuse dripping of sweat from the body. In contrast to the findings of some studies in human subjects, whole-body sweat loss was attenuated with repeated heat exposure. Although sweating rates were slightly higher during exercise after acclimation, a more rapid abatement of sweating during the recovery period after exercise resulted in a significant reduction in overall sweat losses (Table 6.3.2). Similarly, calculated sweat ion losses were 26% lower after acclimation, for the most part a result of a 10% decrease in mean sweat sodium concentration. The more rapid decline in sweating rate during recovery may represent an adaptive mechanism for conservation of water and ions. An increase in respiratory heat loss as a result of humid heat acclimation, evidenced

**Fig. 6.3.4**

Mean ± SE exercise duration during a standardized exercise test (SET) at 50% maximal O_2 uptake ($\dot{V}O_{2\text{ max}}$) in cool, dry (CD; room temperature $[T] = 20^\circ\text{C}$; relative humidity $[RH] = 45\text{--}55\%$) and hot, dry conditions ($T = 32\text{--}34^\circ\text{C}$, $RH = 45\text{--}55\%$) before (HD 0) and after 18 days of heat acclimation (HD 18).

*Significantly different from CD and HD 18;

**significantly different from HD 0 ($P < 0.05$). (Data from Geor et al.⁶⁴)

by an increase in post-exercise respiratory rate, may have partially offset the decrease in cutaneous heat loss during the recovery period.^{63,64}

In summary, horses undergo a number of physiological adaptations to repeated cycles of exercise heat stress that are consistent with a heat acclimation response. These adaptations are evident after 7–14 days and appear to confer a modest improvement in tolerance for exercise in the heat although, similar to

observations in humans,³⁷ this improvement is largely negated when exercise is performed in uncompensable heat stress conditions (high heat and humidity).

The physiologic adaptations associated with acclimatization to heat will diminish in the absence of the thermoregulatory stimulus. In human subjects, the rate of decay is reported to vary from one to several weeks.^{61,62} In physically fit individuals, there is a slower rate of decay of the thermoregulatory adaptations emphasizing the importance of exercise training in augmenting the benefits of heat acclimatization.⁶³ The time course of the decay in thermoregulatory adaptations in horses has not been reported.

Hydration state

Prolonged exercise, particularly when it is performed in hot and humid ambient conditions, can result in large fluid deficits that limit thermoregulatory and cardiovascular function. Studies in humans have demonstrated that dehydration of as little as 2–3% of body weight can impair heat transfer from the body core to the periphery, decrease sweating sensitivity (the increase in sweating rate per unit increase in core temperature), and increase the rate of rise in core body temperature during exertion.^{67–69} Furthermore, dehydration decreases exercise heat tolerance such that fatigue occurs at a lower core temperature when compared to exercise undertaken in the euhydrated state.^{70,71} In horses during 40 min of exercise eliciting 40% $\dot{V}O_{2\max}$, pre-exercise dehydration (4% of body-weight) induced by water withholding or furosemide administration decreased cardiac output and increased pulmonary artery blood and middle gluteal muscle temperatures (by approximately 1°C) when compared to the euhydrated state. Peak sweating rates during exercise were not affected by hydration state.⁷² The authors concluded that the dehydration-induced impairment of thermoregulation was primarily due to a decreased transfer of heat from core to periphery.

The effects of dehydration on thermoregulatory function in horses are magnified during exercise in the heat. During 90 min of treadmill running at 50% $\dot{V}O_{2\max}$ under hot ambient conditions (room temperature 33–35°C; relative humidity 50–55%), progressive dehydration equivalent to 6–8% of bodyweight was associated with decreases in stroke volume, cardiac output, and sweating rate, and higher blood, muscle, and rectal temperatures when compared to trials in which hydration state was maintained by the administration of oral fluid equivalent to approxi-

mately 85% of the incurred sweat fluid losses.²⁷ In summary, pre-exercise dehydration or the progressive dehydration incurred as a result of sweat fluid losses during prolonged exercise will impair thermoregulation in horses. This impairment of thermoregulation will be reflected by a more rapid rise in body temperature. Such an exacerbation of hyperthermia would be expected to reduce exercise capacity due to earlier attainment of a critical upper limit in core and brain temperatures.

Old age

Almost all the studies that have addressed thermoregulatory responses to exercise in horses have used young subjects.^{6,13,19,38,57} As a result, there are very limited data on the effects of age on thermoregulatory function during activity in older horses. McKeever and co-workers⁷³ demonstrated that the older horses had higher heart rates and attained a core body temperature of 40°C in approximately 50% less time than younger mares when both groups were required to work at the same absolute work output (1625 watts). Despite their inability to dissipate heat load at a similar rate during exercise when compared to the younger mares, the older horses had similar heart rates and core temperatures 10 min post-exercise.^{73,74}

In human studies, older subjects have been shown to have lower total body water and plasma volume and, as a result, will have a smaller fluid volume that is available to produce sweat.^{75,76} There is some suggestion that older subjects are chronically hypohydrated and that these age-related alterations in fluid and electrolyte status will add to an age-related reduction in thermoregulatory capacity. However, based on the limited data available regarding older horses, it appears that the fluid shifts during exercise are of similar magnitude in the aged and young horses.^{73,74}

The sweating rate in older horses has been determined to be higher when working at the same absolute work intensity as younger mares. However, it would appear that this increased sweating rate did not improve the ability of the older horses to dissipate heat when compared to their younger counterparts. This could suggest that higher sweating rates occurred in the presence of lower skin blood flow in older versus younger horses.⁷³ An apparent decline in maximal heart rate and stroke volume measured in older horses may contribute to this reduced heat dissipatory capacity.^{77,78} While skin blood flow measurements were not undertaken in these studies, impaired cutaneous blood

flow has been demonstrated in older human subjects in response to exercise.⁷⁶ The mechanism for this age-related change in skin blood flow has not been determined. Chronic hypohydration and a concomitant decrease in plasma volume have been suggested as a contributing factor for differences in thermoregulatory capacity in older versus young human subjects.^{75,76} In one study, pre-exercise plasma volume was lower in old when compared to younger mares.⁷³ This relative hypovolemia could impair the thermoregulatory responses of older horses.⁷⁴

Recommendations for preparation for exercise or competition in hot conditions

Although it is not possible to eliminate adverse effects of environmental conditions on exercise performance, there is evidence from human and animal (including equine) studies that a thorough exercise training program together with a subsequent period of acclimatization will mitigate the impact of the environment. Careful planning is needed for horses transported from a temperate to a hot, humid climate, and then required to train and compete in the hot environment. These horses should attain a high level of event-specific fitness before the trip, and be given adequate time for acclimatization to exercise in the hotter conditions. The hair coat should be clipped to facilitate cutaneous heat loss.⁷⁹ For the 1996 Olympic Games in Atlanta, Georgia, the International Equestrian Federation recommended that horses arrive approximately 3 weeks in advance of the events. This recommendation was based on research studies^{38,63–66} that demonstrated physiological adaptations consistent with heat acclimatization after 14 consecutive days of exercise conditioning in heat and humidity. Minimal training was recommended in the first week, to allow horses to recover from the effects of prolonged transportation. There should not be the expectation of conducting the major portion of a horse's conditioning during this period.

Initially, only light exercise should be undertaken during the heat of the day, with harder workouts performed during cooler periods, e.g. early morning. Subsequently, there should be a gradual increase in the duration and intensity of exercise performed in the heat. It is important that some exercise be performed at the intensity required of the horse during competi-

tion. Careful clinical monitoring is required to assess successful adaptation to the environment. Collection of detailed clinical data should begin 1–2 weeks before travel to the hotter climate. These data will provide baseline information against which to compare clinical responses during the initial days of training in the hot conditions. In addition, evaluation of this information will provide an objective assessment of how well the horse is adapting to the hot environment. Water and feed intake should be measured on a daily basis. Heart rate, respiratory rate, and rectal temperature should be recorded before and after training sessions. The intensity of work efforts can be estimated by use of a heart rate monitor. Daily weighing is useful for estimation of the extent of fluid losses associated with travel and training.

Assuming similarity in the duration and intensity of exercise bouts undertaken in both cool and hot ambient conditions, the exercise-induced increase in rectal temperature will serve as an indicator of the added thermal burden associated with the hotter conditions. The monitoring of post-exercise rectal temperature is critical to early detection of heat exhaustion and other heat illnesses. As there is a lag in the rise in rectal temperature, particularly after heavy exercise in hot and humid conditions, it is important to continue to monitor rectal temperature during the first 5–10 min after exercise. A rectal temperature that exceeds 42°C (108°F) indicates the need for immediate and vigorous cooling. Hyperthermia of this magnitude also may signal the need for a reduction in the intensity or duration of exercise in the hot ambient conditions. There may be an increase in resting respiratory rate following the transition from cool to hot ambient conditions. This increase reflects the thermoregulatory role of the respiratory system. Similarly, the post-exercise respiratory rate is typically 30–40% higher following exercise in hot ambient conditions than in cool to moderate environments. In very hot and humid conditions (temperature of 33–35°C, relative humidity 60–75%), particularly when the horse is not cooled by the application of cold water, the respiratory rate may remain increased (up to 80–100 breaths/min) for 20–30 min after exercise. Persistence of tachypnea after the horse is placed in a cooler environment or is vigorously cooled may indicate that body temperature is still markedly elevated.

Maintenance of fluid and electrolyte balance is important for successful adaptation to a hot environment. Indeed, studies in humans have demonstrated that any thermoregulatory benefits derived from heat acclimation are overwhelmed by the elevated heat

stress imposed by hypohydration.^{61,71} The first concern is the fluid deficit resulting from the period of transport to the new location. Even when access to food and water is maintained during travel, horses typically incur a substantial loss of weight (approximately 3 kg/h of transport). Fluid losses will be higher during road transport in hot weather. As many horses drink poorly during the initial days in a new environment, this dehydration may persist for 3–4 days after arrival. Oral or intravenous fluid support is often provided to recently transported horses in an attempt to hasten correction of fluid balance. Dehydration of as little as 2–3% of bodyweight not only impairs thermoregulation and exercise performance but also prevents the expansion in plasma volume that typically occurs during the early phases of heat acclimatization. Thus, it is imperative that dehydration is corrected before commencement of training in the heat.

Given the increased fluid losses associated with a period of exercise training in a hot climate, an increase in daily water consumption is to be anticipated. A 30–50% increase in 24 h water consumption was observed in horses undergoing heat acclimation; this change occurred within 10 days of the start of heat exposure and largely reflected an increase in water consumption in the 1 h period after exercise.⁶³

Electrolyte or salt supplementation is recommended for horses training and competing in hot climates. Fat supplementation has been advocated as a means of reducing thermal stress in horses exercising in hot

climates. A high-fat diet (8–10% on a total diet basis) may reduce the heat generated by colonic fermentation when compared to a more traditional diet that is higher in roughage.⁵ In addition, lower roughage intake may decrease 'bowel ballast', which may be beneficial during high-intensity exercise. However, the actual effects of these dietary manipulations on thermal load in exercising horses have not been quantified. A limited number of studies have demonstrated that fat supplementation has no effect on fluid and electrolyte balance in horses performing daily exercise in hot conditions (mean daily temperature of 29.2°C).^{80,81}

Regardless of the level of training and acclimatization to hot conditions, it should be recognized that it is possible to overwhelm the thermoregulatory capacity of horses with a high level of physical conditioning when exercise is prolonged and/or there are adverse environmental conditions that will impair heat dissipatory mechanisms. Under such conditions, seeking shade at stops to reduce solar heating, using fans to improve convective cooling, and frequent, copious application of cool water will reduce the rate at which heat is stored. The extent of fluid losses can also be ameliorated if horses are trained to drink fluids at rest stops during competition. Close monitoring of horses exercising under hot conditions is essential to ensure early recognition of hyperthermia, which, left unattended, can progress to the development of heat exhaustion and heat stroke.

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Hematologic and serum biochemical responses to exercise and training

Janene K. Kingston

Blood consists of many components that play an essential role in supporting the increased metabolic rate during exercise by transporting oxygen, water, electrolytes, nutrients, and hormones to working muscles. In addition, carbon dioxide and other waste products produced during exercise are removed from muscle by the circulation. Finally, blood components are important in buffering the acid–base changes associated with exercise. The cellular components of blood include erythrocytes, leukocytes, and platelets, whereas the plasma component is made up of water, electrolytes, plasma proteins, and various hormones and enzymes.

Over the years, evaluation of the hemogram and plasma or serum biochemistry has been used to assess the health status or function of a range of body systems in the athletic horse. These tests are commonly used to assess fitness and performance potential, as well as to investigate poor performance in horses. However, there is a wide degree of variation in values of various blood constituents depending on, among other things, whether a horse is at rest or is exercising when the sample is collected. Additionally, the training status of a horse, as well as sampling techniques, can directly affect the hemogram and serum or plasma biochemistry. It is important that the veterinarian is aware of how such factors may impact test results. In this chapter, those factors that can affect the interpretation of hematology and serum or plasma biochemical profile will be detailed.

Methods

Blood samples should be collected using appropriate equipment and techniques. The jugular vein is com-

monly used for blood sampling and blood is collected into evacuated glass collection tubes. For routine hematology, blood samples should be collected into tubes containing ethylenediaminetetra-acetic acid (EDTA) as an anticoagulant. Given that EDTA can cause a pseudothrombocytopenia,¹ an additional sample may be collected into sodium citrate to ensure an accurate assessment of platelet numbers. Samples for serum or plasma biochemistry can be collected into tubes that do not contain an anticoagulant (serum) or into tubes containing lithium or ammonium heparin (plasma). Some laboratories prefer serum to plasma samples; it is therefore wise to ascertain the laboratory's preference prior to collection of blood samples. It is also important to note that samples collected into EDTA are unsuitable for plasma biochemistry measurements whereas samples collected into heparin are unsuitable for hematology. In addition, if plasma glucose or lactate values are to be measured, blood should be collected into tubes containing sodium fluoride/potassium oxalate as an anticoagulant.

When assessing hematology, the technique of collection, the horse's attitude and degree of excitement, relationship to feeding, and time of day can all have a profound effect on values. In addition, storage of blood samples overnight may result in a slight elevation of hematocrit and mean cell hemoglobin (MCH), probably due to enlargement of erythrocytes.² Storage of blood samples can also result in alterations in plasma or serum biochemistry. Therefore, it is important to standardize collection and handling techniques as much as possible.

The temperament of the horse and the technique used by the sample collector can have an important effect on both the erythrocyte and leukocyte values.^{3–5} Excited horses that resist venepuncture and move

about forcefully have higher erythrocyte and leukocyte counts.⁴ This may relate to the time taken to collect a blood sample in more fractious horses. It takes 30–60 s to mobilize erythrocytes from the spleen following the administration of intravenous epinephrine (adrenaline).⁶ It is likely that samples collected within 30 s from less excited horses have fewer or less marked alterations in the hemogram.^{4,7} At the other end of the spectrum, it is possible that very placid horses have values for red cell indices that are lower than the mean for values for the breed.⁸ Therefore, it is important to note the attitude of the horse during blood collection so that the results can be correctly interpreted.

Other important factors that affect the hemogram and serum or plasma biochemistry are the diet and time of feeding. For example, in the hours after ingesting a hay meal there is a substantial increase in hematocrit and plasma protein concentration (Fig. 7.1.1).⁹ These changes are a result of increased salivary fluid production⁹ and fluid shifts from the circulation to the gastrointestinal tract.^{10–12} Furthermore, feeding a large concentrate meal results in a 12% increase in plasma protein concentration with significant reductions in plasma volume.^{12,13} Therefore, one should avoid collecting blood samples within 3 h of feeding a large concentrate meal or hay ration, or at least ensure that samples are collected at the same time each day and that feeding status is noted to allow for appropriate interpretation of values.

Following exercise it takes 1–2 h for hemogram changes to return to pre-exercise values. If blood

samples are collected in the afternoon after morning exercise, there is a higher proportion of neutrophils and higher leukocyte numbers than in blood samples collected in the morning before exercise.¹⁴ However, there does not appear to be a significant difference between hemograms for samples collected from resting horses either in the morning or afternoon.¹⁵ It is therefore recommended that blood samples are collected either prior to exercise in the morning or on days when horses do not exercise to avoid exercise-induced effects on the hemogram.

Hematology

Structure and function

Erythrocytes

Erythrocytes are anucleate cells that normally circulate for several months in blood. Their primary purpose is to carry hemoglobin, a heme-containing protein that accounts for 95% of the total protein in erythrocytes. Erythrocyte functions include oxygen transport to the tissues, carbon dioxide transport to the lungs, and hydrogen ion buffering, all of which are interrelated.

Oxygen transport

Oxygen is carried in the blood in two forms. Most oxygen is carried in combination with hemoglobin but some is carried as dissolved oxygen. Under normal conditions in arterial blood, 0.3 mL of dissolved oxygen is carried per 100 mL of blood.

The amount of oxygen transported by hemoglobin is much greater than that carried in the dissolved form. Hemoglobin is a tetrameric protein consisting of four polypeptide globin chains, each of which contains a heme prosthetic group. Each hemoglobin tetramer can bind four molecules of oxygen when fully saturated. Under normal circumstances, the presence of hemoglobin-containing erythrocytes increases the oxygen-carrying capacity of blood to approximately 70 times more than that which would be transported dissolved in plasma.¹⁶ The oxygen molecule combines loosely and reversibly with the heme portion of hemoglobin. The partial pressure of oxygen (PO_2) affects the quantity of oxygen bound with hemoglobin. When the PO_2 is high, as in the pulmonary capillaries, oxygen binds with the hemoglobin, but when the PO_2 is low, as in the tissue capillaries, oxygen is released from the

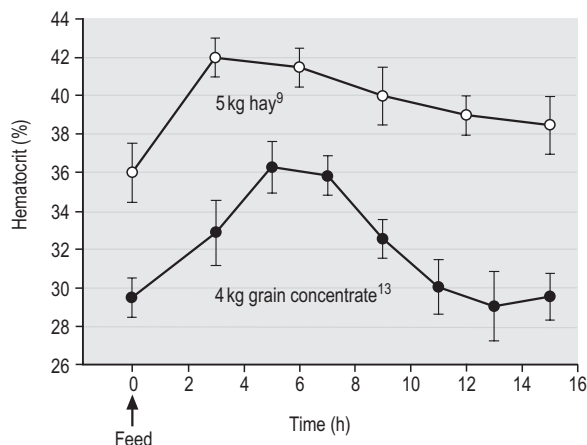


Fig. 7.1.1

The effect of a single feed of 5 kg of hay⁹ or 4 kg of grain concentrate¹³ on the hematocrit.

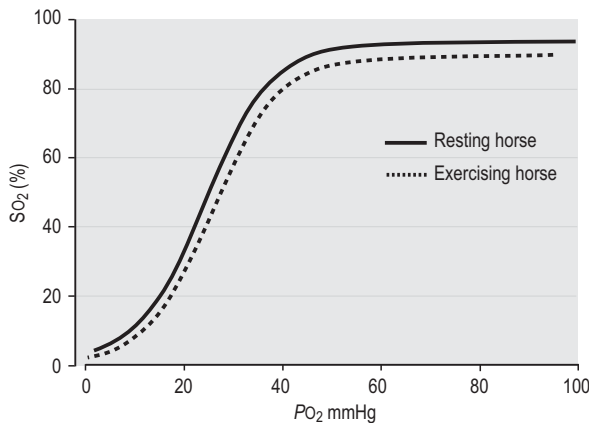


Fig. 7.1.2

Oxygen–hemoglobin dissociation curve in resting (solid line) and heavily exercising (dashed line) horses. PO_2 , partial pressure of oxygen; SO_2 , percentage saturation of hemoglobin. (Adapted from Lekeux.¹⁷)

hemoglobin. There is a progressive increase in the percentage of hemoglobin that is bound with oxygen as PO_2 increases, yielding the oxygen–hemoglobin dissociation curve (Fig. 7.1.2).¹⁷ The curved nature of the oxygen–hemoglobin dissociation curve has several physiological advantages. The flat upper portion means that, even if the alveolar gas PO_2 falls somewhat, loading of oxygen will be little affected. In addition, as the red cell takes up oxygen along the pulmonary capillary, a large partial pressure difference between alveolar gas and blood continues to exist when most of the oxygen has been transferred, hastening diffusion. The steep lower portion of the dissociation curve means that the peripheral tissues can withdraw large amounts of oxygen for only a small drop in capillary PO_2 . This maintenance of blood PO_2 assists the diffusion of oxygen into the tissue cells.¹⁶

The oxygen–hemoglobin dissociation curve can be displaced such that the affinity for oxygen is altered. Factors that shift the curve include changes in carbon dioxide concentration, blood temperature, blood pH, and the concentration of 2,3-diphosphoglycerate (2,3-DPG). Changes in blood carbon dioxide and hydrogen ion concentration have a significant effect in enhancing oxygenation of blood in the lungs and enhancing the release of oxygen from the blood to the tissues through the Bohr effect.

Carbon dioxide transport

Carbon dioxide is transported in blood as either dissolved carbon dioxide (5%), bicarbonate ions (70–90%), or carbamino compounds (5–10%). The majority of carbon dioxide is transported as bicarbonate ions. This is because erythrocytes have a high activity of carbonic anhydrase, an enzyme that catalyzes the reaction between carbon dioxide and water. This reaction makes it possible for water to interact with large quantities of carbon dioxide, enabling transport of carbon dioxide from the tissues to the lungs in the form of bicarbonate. This is particularly important during exercise as working muscles release large quantities of carbon dioxide.

As described earlier for the Bohr effect, the partial pressure of carbon dioxide and pH can loosen the binding of oxygen with hemoglobin. Conversely, oxygen can also act to displace carbon dioxide and hydrogen ions from hemoglobin; this is termed the Haldane effect. Both the Bohr and Haldane effects can be explained by the fact that deoxyhemoglobin is a weaker acid than oxyhemoglobin. Hence, deoxyhemoglobin more readily accepts hydrogen ions liberated by the dissociated form of carbonic acid at the tissue level when carbon dioxide is released into the blood from the tissues. This allows more carbon dioxide to be transported in the form of bicarbonate ions. At the same time, the association of hydrogen ions with hemoglobin lowers the affinity of hemoglobin for oxygen, causing a shift of the oxygen–hemoglobin dissociation curve to the right and facilitating the unloading of oxygen at the tissue level.¹⁶

Leukocytes

Leukocytes have a primary role in immune function. There are normally six different types of leukocyte found in the circulating blood: neutrophils, eosinophils, basophils, monocytes, lymphocytes, and occasional plasma cells. In resting blood samples, the white cell count does not reflect the total intravascular pool, as this consists of both the circulating and marginal pools, the latter being sequestered in capillary beds and the spleen.¹⁸ Therefore, any factors that cause mobilization of cells from the marginal pool will increase the numbers of leukocytes in the circulating pool. Such factors include plasma catecholamine and cortisol concentrations. Therefore, recent stress such as transportation or exercise of a horse can alter the white cell count in blood samples.

Platelets

Platelets are anucleate cell fragments derived from megakaryocytes in the bone marrow that normally circulate in the bloodstream for 4–5 days. Platelets interact with the endothelium and circulating coagulation factors in the maintenance of normal hemostasis. In addition, platelets are also thought to be involved in inflammatory and immunological processes.¹⁹ Platelets are also sequestered in the spleen and are subject to increases in circulating numbers with splenic contraction. Platelets are also subject to activation following injury of the endothelium, through inappropriate handling *in vitro* and potentially through shear stress in the vascular system. For the exercising horse, each of these factors is a consideration when evaluating both platelet numbers and activation status.

The normal resting hemogram

For the normal resting hemogram, the normal range for an individual horse is quite narrow, whereas there are much broader ranges across horse breeds, with further variation due to age and state/type of training. The red cell indices show the greatest variation amongst breeds. This relates to factors such as plasma volume expansion seen in endurance-trained animals but not in Thoroughbred or Quarter Horse race horses. In addition as mentioned earlier, the temperament of a horse can significantly affect red cell indices.

Hematologic responses to exercise

Erythrocyte indices

Exercise has variable effects on the hemogram depending on work intensity (Fig. 7.1.3).^{20–28} Exercise generally results in mobilization of splenic erythrocytes and, therefore, increases the oxygen transport capacity. The extent of the potential increase in circulating red cell mass is quite remarkable, as the spleen has the capacity to store up to 50% of the total red blood cell pool.²⁹ The release of splenic erythrocytes is under the influence of catecholamines. Both the intensity and duration of exercise are important in determining the magnitude of the catecholamine response.³⁰ The extent of the increase in hematocrit is a function of exercise intensity, with a linear relationship between

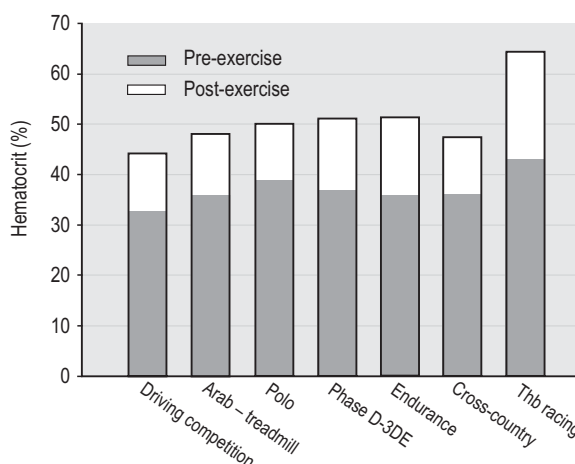


Fig. 7.1.3

The effect of different types of exercise including the cross-country phase of driving trail competition,²¹ low-intensity treadmill exercise,²⁸ during a polo match,²⁶ phase D of a three-day event,²⁵ an 80-km endurance ride,²⁴ the cross-country phase of a three-day event,²² and Thoroughbred (Thb) racing²⁰ on the hematocrit.

hematocrit and speed,^{23,31} up to a maximum hematocrit of approximately 60–65%.⁷ There are, however, variations in splenic capacity in association with breed of the horse as well as age. Draught horses have lower relative splenic weights compared to Thoroughbred horses³² and it appears that splenic capacity alters in response to increasing age (from 1 to 3 years) in Trotters.^{31,33}

While the majority of the increase in hematocrit during high-intensity exercise is attributable to splenic contraction, exercise-induced fluid shifts also play a role. For horses performing moderate short-term incremental exercise there is a 5–10% decrease in plasma volume.³⁴ However, given the substantial fluid losses incurred during prolonged endurance exercise, it is likely that reductions in plasma volume play a greater role in changes in hematocrit observed in endurance exercise.

In association with the increases in hematocrit are increases in the erythrocyte count and hemoglobin concentration. As a consequence of the increase in hemoglobin concentration there is an increased oxygen transport capacity, an important factor in the horse's high aerobic capacity.³⁵ Indeed, studies of splenectomized horses have shown a marked reduction in exercise capacity.^{29,36} However, the increase in blood

viscosity associated with exercise and increased hematocrit^{37–39} likely reaches a point that offsets improved oxygen-carrying capacity. This probably accounts for the marked reduction in performance in horses with red cell hypervolemia.³¹

Other erythrocyte changes associated with high-speed exercise include small increases in mean corpuscular volume (MCV) and decreases in MCH and mean corpuscular hemoglobin concentration (MCHC). In addition, erythrocytes in blood samples obtained after exercise are more resistant to osmotic stress.⁴⁰ However, although Smith et al⁴⁰ did not detect any differences in erythrocyte deformability, Geor et al³⁹ detected reduced erythrocyte deformability during exercise. The difference between these two studies may be attributable to the techniques used to assess erythrocyte deformability. Deformability of erythrocytes is considered to be the major determinant of resistance of blood to flow in the microcirculation,⁴¹ with reduced erythrocyte deformability potentially increasing blood flow resistance. However, horses are remarkably resistant to changes in blood viscosity associated with increases in hematocrit, likely because of changes in red cell deformability.^{42,43}

It has been reported that there are large numbers of echinocytes released during exercise.⁴⁴ However, other studies of horses exercising for shorter durations at higher intensity have found the concentration of echinocytes to be quite low.^{39,40} These differences might relate to exercise intensity and duration or possibly to the breed of horse. The significance of echinocytes is unclear; however, for short-duration, high-intensity exercise, they are probably not of physiologic importance in changing oxygen delivery.⁴⁰ Their role in prolonged low-intensity exercise is yet to be defined.

There is a four-fold increase in oxygen extraction from blood during exercise. This increase in extraction ratio is facilitated by a rightward shift of the oxygen–hemoglobin dissociation curve. The shift occurs because of acidosis, hypercarbia, and hyperthermia in the local muscle environment.⁴⁵ There are also increases in the levels of 2,3-DPG during exercise.⁴⁶ This causes a rightward shift in the oxygen–hemoglobin dissociation curve, further enhancing the release of oxygen from hemoglobin to the tissues. Low blood PO_2 stimulates erythrocyte glycolysis and the formation of 2,3-DPG. The increased oxygen extraction during exercise also makes blood more effective in the transport of carbon dioxide due to the presence of increased amounts of deoxyhemoglobin for formation of carbamino products.⁴⁵

Leukocytes

There are significant differences in the response of leukocytes to exercise of differing intensities and duration (Fig. 7.1.4). Following high-intensity exercise, significant increases in leukocyte numbers are not seen. Immediately after galloping, there is a change in the neutrophil:lymphocyte ratio, but little change in the total leukocyte count. There is a transient lymphocytosis with a decrease in the neutrophil:lymphocyte ratio.^{20,47} These changes are likely secondary to catecholamine release and splenic contraction. At 3 h after exercise there is an increase in the neutrophil:lymphocyte ratio, caused by an increase in neutrophils and decrease in lymphocytes due to increased plasma cortisol concentrations. However, the neutrophil:lymphocyte ratio returns to normal by 6 h after exercise.²⁰

In contrast to high-intensity exercise, endurance exercise is associated with a leukocytosis, resulting from a neutrophilia and lymphopenia.^{24,48} This is probably due to an increase in circulating corticosteroids,^{49–51} with speed significantly affecting the extent of the neutrophilia and lymphopenia. Horses that complete an endurance ride at a faster speed have a higher neutrophil:lymphocyte ratio than slower horses.²³ Additionally, Carlson et al⁵² showed that exhausted endurance horses had a significant left shift

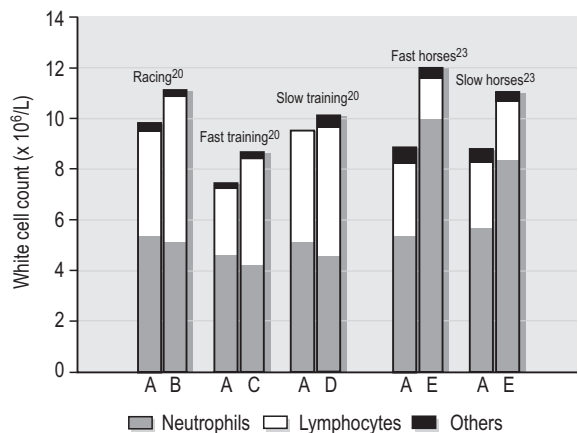


Fig. 7.1.4

The effect of different types of exercise on the leukocyte numbers. A, resting; B, immediately after racing; C, immediately after fast training (11–12 m/s); D, immediately after slow training (13 m/s); E, immediately after a 160 km endurance ride. (Data from Snow et al²⁰ and Rose²³)

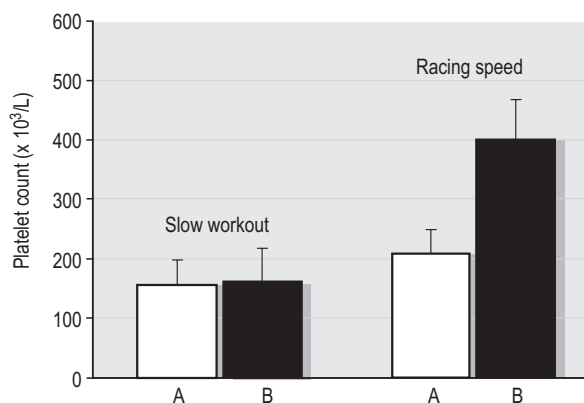


Fig. 7.1.5

The effect of exercise on platelet numbers. A, before exercise; B, after exercise. (Data from Johnstone et al.⁵⁵ and Bayly et al.⁵³)

in the neutrophils when compared to clinically normal horses, even though the total white cell count was similar to that reported by Rose.²³ For samples collected the day after an endurance ride, a slower group of horses still had a significantly increased white cell count compared to a group of fast horses. However, this could be associated with the time the samples were collected: 14 h versus 21 h post-exercise for the slow and fast horses, respectively.²³

Platelets

Similar to erythrocytes, exercise has a variable effect on platelet numbers that appears to be exercise intensity-dependent (Fig. 7.1.5). High-intensity exercise results in significant increases in circulating platelet numbers⁵³ whereas moderate exercise does not significantly increase platelet numbers.^{54,55} With regard to platelet activation and aggregability, some studies report reduced platelet aggregability in response to high-intensity exercise;^{53,55} however, others have reported increased aggregability⁵⁶ and activation of platelets.^{57,58} It is likely that increases in the sodium citrate concentration associated with hemoconcentration of blood samples resulted in reduced ionized calcium concentration⁵⁹ and platelet aggregability.^{53,55} No studies have looked at the effect of exercise duration, age, or breed of the horse on the response of platelets to exercise.

Hematologic responses to training

Erythrocyte indices

There has been a large number of studies investigating the response of the hemogram to various types of training. The most common finding for horses undergoing high-intensity training has been significant increases in the resting red blood cell count, hematocrit, and hemoglobin concentration.^{20,60–62} The increased erythrocyte numbers observed in horses training at higher intensities are thought to result from a greater demand for oxygen carriage, which stimulates erythrocyte production. Interestingly, in one study, horses with higher hematocrits (>40%) at the start of training did not show any significant change in red cell indices compared to horses starting with a lower hematocrit.⁶⁰ This highlights some of the problems in trying to interpret the resting hemogram. Variations exist with the demeanor of the horse, as well as time of blood collection. The increased excitability of a horse as it gets fitter could result in elevations of resting red cell indices rather than the training per se and might account for the results reported by Clarkson and others.⁸ Regular monitoring of the hemogram during training has little value for assessing a horse's fitness.

Despite the variability of the resting hemogram, there does appear to be an increase in total hemoglobin and total red cell volume with training. However, due to variability in individual plasma volume, an assessment of post-exercise hematocrit or hemoglobin alone lacks accuracy for determination of the total erythrocyte mass.³³

In Standardbred Trotters, prolonged training can result in an excessive increase in the red cell mass and the phenomenon known as red cell hypervolemia.^{31,63} This results in reduced racing performance and appears to be due to overtraining. These horses have lower oxygen consumption. It has been hypothesized that increased blood viscosity leads to reduced capillary perfusion and inadequate utilization of oxygen by the working muscles. This phenomenon has not been observed in other breeds of performance horse, so it is unclear if it plays a role in overtraining in other breeds. It is also worth pointing out that there have been no prospective studies that have shown that development of red cell hypervolemia during racing and training is accompanied by concurrent loss of performance. It is unknown whether the loss of performance reported in Swedish Standardbred Trotters occurs at the same time as red cell hypervolemia. In a recent

longitudinal study of experimentally induced overtraining in twelve Standardbred geldings, red cell hypervolemia was not a mechanism for loss of performance.⁶⁴ Therefore, further prospective studies are needed to test the hypothesis that red cell hypervolemia is a cause of poor performance.

Endurance-trained horses have lower resting red cell indices than race horses. Similar to race horses, the effects of training on red cell indices are variable. In a group of Standardbred horses, after 7 weeks of low-intensity exercise training there were no significant effects on the resting or recovery hemogram.²⁷ Similarly, in a group of endurance horses (predominantly Arab crosses) there was no change in the resting hematocrit over 12 weeks of training, although quieter horses had significantly lower hematocrits throughout the training period compared to more apprehensive horses. Nevertheless, it is possible that there could be a reduction in hematocrit in endurance horses due to expansion of plasma volume.⁶⁵ It is clear from the results of these studies that hematological measurements are of little value in assessing the fitness or progress of horses during training.

Blood gas transport

Several studies have examined the effect of exercise training on blood oxygen transport properties in horses.^{46,61,66} The results have been variable, although an increase in circulating blood oxygen capacity at rest, and an increase in blood oxygen affinity due to decreases in erythrocyte 2,3-DPG concentration, have been described.⁶¹ With regard to 2,3-DPG concentration, Lewis & McLean⁶⁶ and Lykkeboe et al⁶¹ showed a reduction in red cell 2,3-DPG concentration in response to training, whereas Pelletier et al⁴⁶ showed no change in 2,3-DPG concentration. This could potentially be due to the different training programs used in those studies. A reduced 2,3-DPG concentration could potentially impair unloading of oxygen. However, this has not been further evaluated and detrimental effects on work performance have not been reported.

Leukocytes

The total leukocyte count is unchanged during training of race horses and horses used for endurance racing. In addition, there are no alterations in the proportions of the different leukocytes. While there have been anecdotal reports of alterations in the neutro-

phil:lymphocyte ratio in horses that are overtrained, this was not observed by Tyler-McGowan et al.⁶⁷ However, several horses in that study developed an absolute eosinopenia together with clinical signs of illness. It was hypothesized that eosinophils may be a more sensitive indicator of training stress than other members of the leukocyte series.

Platelets

Although a large number of studies have investigated changes in the hemogram in response to training in horses, no studies have documented platelet numbers during training. Furthermore, given many of the technical problems when working with equine platelets, it is currently unknown if exercise training alters platelet function.⁵⁹

Plasma or serum biochemistry

Muscle-derived enzymes

Creatine kinase (CK) is a relatively muscle-specific enzyme with a plasma half-life of approximately 2 h.⁶⁸ In muscle, CK makes ATP available for contraction by phosphorylation of adenosine diphosphate (ADP) from creatine phosphate. Apart from skeletal muscle, CK activity is also present in gastrointestinal tissue, uterus, urinary bladder, kidney, heart, and thyroid gland.⁶⁹ Increased serum CK activity may be due to muscle damage or to injury to organs containing smooth muscle, and be falsely increased with *in vitro* hemolysis.⁷⁰

Aspartate aminotransferase (AST) has a much longer half-life (approximately 7–8 days)⁶⁸ than CK and is less specific for muscle, being found in most tissues.⁷¹ It is a cytoplasmic and mitochondrial enzyme that catalyzes the deamination of aspartate to form oxaloacetate, which can enter the Krebs cycle. Increases in plasma AST activity may be due to hepatocyte damage, muscle damage, or *in vitro* hemolysis.⁷⁰

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme that catalyzes the conversion of pyruvate to lactate at the end of glycolysis. Activities of LDH are high in various tissues of the body. Therefore, measurements of LDH are not organ-specific. There are five isoenzymes of LDH, which can be separated by electrophoresis.⁶⁹ The isoenzyme LDH5 is the predominant form in skeletal muscle and has a plasma half-

life of less than 6 h.⁷⁰ Increases in LDH activity may be due to hepatocyte damage, muscle damage, or hemolysis.

Liver-derived enzymes

γ -Glutamyltransferase (GGT) is a membrane-associated enzyme that catalyzes the transfer of glutamyl groups between peptides and is involved in glutathione reactions. Many cells have GGT activity but biliary epithelial cells, pancreas, and renal tubular cells are classically considered to have the greatest activity.⁷⁰ The half-life of GGT in horses is approximately 3 days and it is stable in serum for 2 days at room temperature, or 30 days if frozen.⁷² The primary source of increased plasma GGT activity in horses is cholestasis or biliary hyperplasia.

Alkaline phosphatase (AP) catalyzes the hydrolysis of monophosphate esters and is bound to the mitochondrial membrane. Tissues that contain AP include liver, bone, intestine, kidney, placenta, and leukocytes. Increases in the serum activity of this enzyme in adult horses are secondary to induction. Cholestasis and certain drugs will cause production and the release of AP.⁷³

Sorbitol (iditol) dehydrogenase (SDH) is a cytoplasmic enzyme that catalyzes the conversion of fructose to sorbitol. It has a short plasma half-life with blood levels declining within 4 h of transient hepatic necrosis. Its short half-life necessitates analysis within 12 h of collection, or 48 h if the serum is separated from blood and refrigerated.⁷⁴

Glutamate dehydrogenase (GLDH) is found in hepatocytes, renal tissue, brain, muscle, and intestinal cells. Like SDH, GLDH has the highest tissue concentration in the liver and increases of this enzyme in blood can be considered specific for acute liver disease. The half-life for GLDH is 14 h.⁷²

Plasma proteins

Albumin is synthesized by the liver and is the most prominent of the plasma proteins, constituting 35–50% of total plasma protein in animals. It is the most osmotically active plasma protein due to its abundance and small size and accounts for about 75% of the oncotic activity of plasma. A major role of albumin is as a general binding and transport protein.⁷⁵ The plasma concentration of albumin is affected by

changes in plasma water content and intravascular water volume.

Globulins include α -globulins, β -globulins, γ -globulins, and immunoglobulins. Changes in the concentration of globulins generally reflect inflammation and disease, although hydration can also impact globulin concentration.

Fibrinogen is an acute phase protein, the concentration of which increases in response to inflammation. Dehydration can increase plasma fibrinogen concentration.

Changes in plasma or serum biochemistry associated with exercise

Muscle-derived enzymes

Increases in plasma CK, AST^{25,71,74–79} and LDH⁸⁰ activities have been seen in response to exercise. These increases are believed to relate either to overt damage or to a change in the muscle fiber membrane causing a transient increase in permeability.^{80,81} Physiological increases have been shown to occur without any tissue destruction.⁸² The extent of this increase depends on the nature of the exercise.^{71,80} Results of a number of studies suggest that increases in CK activities occur with strenuous exercise,^{71,83,84} whereas with lighter exercise, other studies have shown no significant increase. This implies that exercise intensity could be an important factor. However, there is also evidence to suggest that duration of exercise may be a more important factor.⁸⁰ These variable findings highlight much of the confusion that exists due to the different exercise intensity and duration regimens used, together with varying sampling intervals and the possibility that the studies may have included horses with possible muscular problems. It has been suggested that sampling at least 24 h after exercise may facilitate the differentiation between those animals showing a normal physiological response to exercise and those with an abnormal or pathological response.⁷¹

Liver-derived enzymes

In general, a single exercise bout has minimal effect on the activities of liver-derived enzymes. However,

increases in serum AP activity have been reported in horses during endurance exercise⁷⁸ and in horses competing in three-day event competitions.⁷⁹ It is unclear whether these increases in AP are of skeletal or hepatic origin.

Plasma proteins

During maximal exercise, there is a redistribution of fluid and electrolytes from the vascular compartment to the tissue extracellular fluid spaces, with a corresponding increase in total plasma protein and albumin.^{22,26,38,85–88} The extent of the fluid shift appears to be related to the duration and intensity of exercise. Less dramatic increases are seen in polo horses²⁶ and horses performing submaximal exercise⁸⁵ than in Thoroughbred race horses⁸⁹ and other horses performing maximal exercise.⁸⁵ Increases in plasma protein associated with short-duration exercise return to pre-exercise values within 15–30 min of exercise.^{26,85,86} However, with hot conditions and extensive sweat losses, these fluid shifts may be more dramatic and prolonged.

While acute exercise in horses results in significant increases in total plasma protein concentrations, individual plasma protein fractions increase in a heterogeneous manner, indicating that changes in plasma protein concentrations cannot be simply attributed to changes in plasma water alone.^{22,38} In a study by Coyne et al.,³⁸ plasma albumin concentration increased by 22%, whereas fibrinogen concentration increased 12.5% and increases in the various globulin groups ranged from 25.5% to 60%. This resulted in a 10% decrease in the albumin:globulin ratio compared to resting samples. The mechanisms behind heterogeneous alterations in plasma protein fraction concentrations are unclear but could include compartmental redistribution, accelerated biosynthesis, increased degradation, and bolus release.³⁸ In addition, exercise-induced fibrinolysis could play a role in limiting the increase in fibrinogen concentration.⁹⁰ However, given that many laboratories consider a fibrinogen concentration above 4 g/L to be clinically significant, acute exercise does not appear to increase fibrinogen to this extent.⁹¹

During prolonged, low-intensity exercise, albumin and total plasma protein concentration increases.^{50,51,77,78,92,93} However, despite the increase in plasma protein concentration, there is evidence that plasma volume increases during the initial stages of

prolonged exercise.^{31,94} Although increased plasma volume has been noted in dehydrated horses after 40 min of low-intensity exercise,⁹⁴ it is likely that plasma volume decreases in response to the substantial fluid losses incurred during prolonged endurance exercise.^{24,95,96} Furthermore, those fluid losses result in increases in albumin and plasma protein concentrations that are much greater than those observed in horses performing short-duration exercise, and it takes longer for plasma protein concentrations to return to normal following prolonged endurance exercise. For horses competing in three-day event competitions⁷⁹ and driving trial competitions,²¹ there are similar increases in plasma protein concentration, which remain significantly increased 30 min after exercise.

Changes in plasma or serum biochemistry associated with training

In general, there are few changes in resting biochemical values as a result of training. Although there have been some significant changes in a number of biochemical measurements, results have been variable and inconsistent.^{23,62,67,97,98} The most consistent change has been in indicators of liver function, with serum GGT activity showing the greatest increase.^{67,98,99} In some horses, GGT activity is greater than 100 U/L, but horses have not shown any other evidence of liver disease.⁸ However, in other studies high-serum GGT activities have been associated with poor health and overtraining in some horses.⁹⁹ Some performance horses with decreased performance have had three- to fourfold increases in serum GGT activity with no other laboratory evidence of hepatic disease. The source of the increase in GGT has not been determined and reportedly returned to normal within 30–60 days.¹⁰⁰

Conclusion

There is no doubt that hematology and plasma or serum biochemistry are important tools for assessing the health of athletic horses. However, many factors can influence measurements, as have been outlined in this chapter. Each of these factors needs to be considered carefully when interpreting blood results. Hema-

tology and biochemistry measures are unlikely to provide useful information regarding the fitness or performance potential of horses. However, they will continue to be useful in assessing health and disease

in individual animals, particularly when samples are collected in a standardized manner and are compared to the results of horses of similar breed and training backgrounds.

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Immunological responses to exercise and training

David W. Horohov

It is a common belief that habitual exercise provides an individual with enhanced protection from disease. A review of the scientific literature, however, reveals seemingly paradoxical reports on the effect of exercise on the immune system.^{1–3} More than 30 years of research on this topic have made it clear that the effects of exercise on the immune system are complex and dependent upon multiple factors, including:

- the immune function or characteristic being analyzed
- the intensity and duration of exercise
- the timing of the measurement of immune function in relation to the exercise bout.^{1,2,4}

The underlying mechanisms whereby exercise alters immune responses are likewise multifactorial and likely include both neuroendocrine^{5–7} and metabolic factors.^{8–10} Although the clinical consequences of the exercise-induced immune changes have not been formally identified, there is evidence that high-intensity exercise in humans can lead to an overall impairment of the cellular immune system, resulting in low concentrations of lymphocytes, suppressed natural immunity, suppressed lymphocyte proliferation, suppressed levels of secretory IgA in saliva, and an increased incidence of upper respiratory tract infections.^{8,11–16} By contrast, a regular routine of moderate exercise seems to improve immunological function and increase disease resistance, particularly in the elderly.^{4,17}

The majority of the studies concerning the immunologic responses to exercise and training have focused on humans and laboratory animals. Although the horse provides an excellent opportunity for exercise-based studies, there is a limited amount of information available concerning the effect of exercise on the equine immune system.¹⁸ Most of this review will therefore emphasize those results obtained from human and rodent exercise studies. When possible,

specific information regarding equine exercise studies will also be included.

Immune responses

Most investigations on the effect of exercise on immune function have focused on the innate immune response in both horse and man.^{1,18,19} This initial response to pathogens plays a central role in disease resistance and the development of subsequent adaptive or specific immune responses. The innate immune response includes both cellular and humoral components, although most studies on the effect of exercise on this response have focused on the cellular components: namely, neutrophils, macrophages, and natural killer (NK) cells.^{18,19} The essential characteristic of the innate immune response is that it does not exhibit specificity for the invading organism. Thus the induction of an innate immune response does not require prior exposure to the invading organism, nor is it augmented by repeated exposure to the same organism. The innate immune response plays an important role in prompting the adaptive immune response as well as providing valuable time for specific adaptive responses to develop.

The adaptive immune response is likewise mediated by both humoral and cellular components. The humoral response consists of antigen-specific antibodies produced by B cells and plasma cells. The cellular response is composed of effector T cells such as cytotoxic T-lymphocytes (CTLs) that exhibit both antigen specificity and the phenomena of immunological memory. The regulation of the adaptive immune response is mediated by T helper (Th) cells that produce various cytokines, small hormone-like proteins that control the growth, differentiation, and activation

state of various cells of the immune system.²⁰ Together, these responses provide the functional capacity to protect an animal completely against a particular pathogen.

Functionality of the innate immune response is assessed *in vitro* by measuring the phagocytic or oxidative metabolic activity of macrophages and neutrophils^{21–24} and the cytotoxic activity of NK cells.^{25–28} The adaptive immune response is assessed *in vitro* by the ability of lymphocytes to proliferate^{4,29–31} or produce cytokines in response to various mitogens and specific antigens;^{4,30,32} and *in vivo* by measuring antibody responses.^{30,33–35}

Exercise intensity and immune function

An acute exercise bout or test is the most frequently used method for exercise immunology studies.^{3,36,37} Even with this approach the overall response depends on many factors, including the intensity, duration, and mode of exercise; plasma concentrations of hormones and cytokines; physiologic changes in the body including temperature, blood flow, and hydration status; and the overall fitness of the subject.³⁶ In general, acute exercise bouts of moderate duration (<60 min) and intensity (<60% $\dot{V}O_{2\max}$) are associated with fewer perturbations to the immune system than prolonged, high-intensity sessions in humans^{36,38,39} and horses (Table 7.2.1).^{23,24,40} Conditioning of elite human athletes may or may not alter exercise-induced changes in immune function, and may be sport-dependent.^{41,42} Studies of unconditioned horses have shown decreased immune responses after a single^{43,44} and multiple bouts of intensive exercise.³⁰ Other

studies involving conditioned horses found decreased lymphoproliferative responses following treadmill exercise⁴⁵ or racing²⁹ and impaired neutrophil function after endurance riding.^{46,47} Long-term exercise training of low intensity appears to improve some measures of human immune function,⁴⁸ whereas overtraining is associated with decrements in some immune measures (granulocyte oxidative burst, NK cell activity, lymphocyte proliferation, the production of cytokines in response to mitogens, nasal and salivary immunoglobulin A levels) and increases in others (granulocyte and monocyte phagocytosis, and inflammatory cytokine production).^{8,13–16} Interestingly, overtraining in humans is associated with a decrease in maximum exercise-induced adrenocorticotrophic hormone (ACTH) and, to a lesser degree, cortisol and free plasma catecholamines.⁴⁹ No similar alterations in stress hormone production have been seen in overtrained horses,^{50,51} although the possibility of immunological alterations in overtrained horses has not been addressed.¹⁸

The stress response

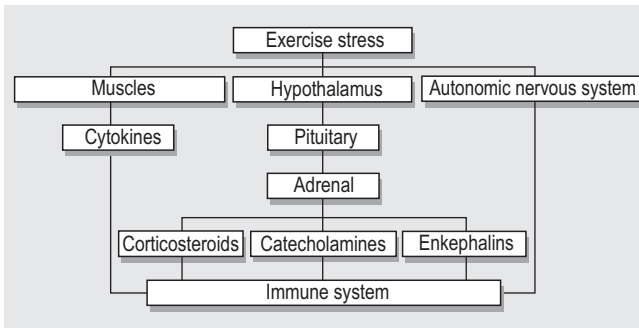
The physiologic and immunologic consequences of intensive exercise parallel the effect of other physical and psychological stressors.^{52–54} It should be noted that exercise stress is not a single homogeneous stressor. Rather, it is actually a culmination of several different stressors, including physiologic, environmental, social, and psychological stressors.^{53–55} Stress has been defined, in general, as an abnormal or extreme adjustment in the physiology of the animal to cope with adverse effects of its environment or management.^{56,57} Initial theories attempting to identify a common non-specific stress response pathway (e.g. Hans Selye's general adaptation syndrome) have been discounted and instead four separate pathways have been identified: the hypothalamic–pituitary–adrenal (HPA) axis, the autonomic nervous system, extra-adrenal pathways, and extraneuronal production of cytokine mediators (Fig. 7.2.1).⁵⁸ Although products of each of the pathways appear to be involved in stress-induced immunomodulation, the HPA axis and its production of cortisol and catecholamines has been the most intensively studied stress pathway in terms of exercise-induced immune modulation (Fig. 7.2.2).^{54,59}

Cortisol represents the chronic response to stress whereas catecholamines represent the acute stress response.^{60,61} Cortisol, the primary biologically rele-

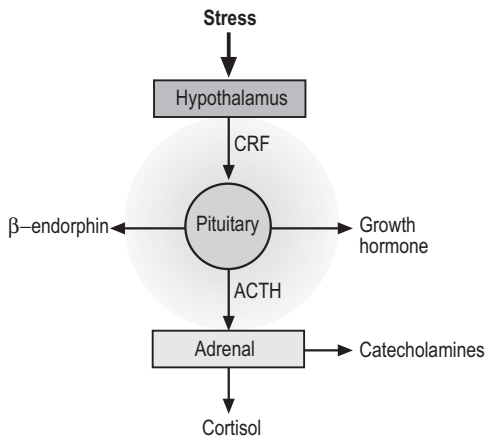
Table 7.2.1 Summary of exercise intensity effects on immune functions¹⁸

Immune function	Moderate exercise	Intense exercise
LAK/NK lysis	↑	↓
Lymphoproliferation	↓	↓
Neutrophil function	↓	↓
Macrophage function	↓	↓
Proinflammatory cytokines	↑	↑
Antibody levels	↑	↓

LAK, lymphokine-activated killer; NK, natural killer.

**Fig. 7.2.1**

Exercise effects on the immune system. Exercise can affect the immune response either directly via the production of various cytokines by muscle cells, or via hormone produced via the hypothalamus–pituitary–adrenal axis.

**Fig. 7.2.2**

Hypothalamus–pituitary–adrenal (HPA) axis. The perception of the stressor signals corticotrophic-releasing factor (CRF) production by the hypothalamus, which causes the pituitary to produce adrenocorticotrophic hormone (ACTH) and β -endorphin. ACTH stimulates cortisol production by the adrenal glands, which also produce catecholamines.

vant corticosteroid of humans and horses, is a cholesterol derivative produced by the adrenal cortex under the direction of ACTH produced by the pituitary.⁶² Production of ACTH is regulated by corticotropin-releasing factor (CRF), which is produced by the hypothalamus.⁶³ Cortisol production in humans and horses has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir in the evening.^{64–66} This rhythm can be disrupted by physical and psychological stress.^{65,66} Intensive exercise is likewise associated with an increase in plasma cortisol in both human and horse.^{54,67,68} Therefore it is tempting to ascribe exercise-induced changes in immune function to increases in plasma cortisol, because corticosteroids are known to be potent anti-inflammatory agents and to have dramatic effects on immune cell function (see below).⁶⁹

Catecholamine production by the nervous system is another important component of the stress response.^{59,70} Catecholamines, such as epinephrine (adrenaline) and norepinephrine (noradrenaline), are derivatives of tyrosine produced by sympathetic nerve endings and the adrenal medulla.⁷¹ They mediate a multitude of physiologic changes that broadly encompass the ‘fight or flight’ response to stressors.^{57,72} Catecholamines exert their effects within minutes of their induction and are considered to represent the active response to stress.⁷³ Catecholamines mediate their effect through the interaction with adrenergic receptors found on the surface of various cells, including lymphocytes.^{74–76} A number of immunomodulatory effects of catecholamines have been described (see below).

It has been suggested that stress-induced impairment of either innate or specific immune responses opens a ‘window of susceptibility’ to infectious agents.⁷⁷ This is likely due to subtler changes in immune function rather than complete immune suppression.^{78–85} These subtle changes in lymphocyte function could account for aberrant responses to vaccination seen in stressed individuals^{86,87} and the increased incidence of upper respiratory tract infections in human athletes.^{88–91} Although the precise mechanism responsible for the increase in disease remains uncertain, a number of in vitro correlates have been identified.

Exercise-induced leukocytosis

One of the most consistent effects of exercise on the immune system is the leukocytosis seen after strenuous exercise in humans,^{21,92–95} as well as horses.^{96–98} As with the other effects of exercise on immune function, the magnitude of the leukocytosis increases as the intensity and duration of exercise increase.⁹⁴ The leukocytosis observed following acute intense exercise

is biphasic in nature, characterized by an initial increase in lymphocyte numbers followed by an increase in neutrophil and monocyte numbers.^{93,95,99} Lymphocyte numbers have been shown to return to resting levels in as short as 1 h after exercise, whereas neutrophils are slower in returning to resting values and, considering the fact that lymphocyte numbers decline rapidly, are responsible for the increased neutrophil to lymphocyte ratio reported in most studies.^{95,96} Lymphocyte numbers have been shown to drop below baseline values in human and equine subjects during the recovery phase following intense exercise creating a lymphopenia,^{61,100} and this effect has been attributed to post-exercise production of cortisol.⁷⁷

In addition to the increase in lymphocyte numbers in response to exercise, it has been shown that exercise has an effect on lymphocyte subpopulations. The majority of the work has concentrated on the changes in circulating numbers of CD4⁺ and CD8⁺ T-lymphocytes.^{39,101–105} Most Th cells are CD4⁺ while CTLs are CD8⁺. In one study with a limited number of horses, CD4⁺ T cell numbers were shown to decline following intense exercise, with no change in CD8⁺ numbers.¹⁸ In related studies using human subjects, CD4⁺ and CD8⁺ T cell numbers have been shown to increase, decrease, or remain stable following exercise.^{39,103–105} In general, these results indicate that exercise-induced lymphocyte subset reduction is transient and dependent upon exercise intensity and duration with high-intensity exercise tending to decrease the number of CD4⁺ T cells.^{12,39,103}

The number of circulating NK cells, identified by the presence of the CD16 cell-surface antigen, has been shown to increase following exercise in humans.^{12,28,39,106–108} The effect of exercise on NK cells in the horse is unknown, because equine NK cells are difficult to identify. Several markers that selectively bind to the equine NK cell have been identified and may provide the answer to this and many other questions regarding equine NK cells.^{109,110} Equine lymphokine-activated killer (LAK) cell activity, which appears to be distinct from NK cell activity,^{111,112} has been shown to increase following exercise,¹¹³ although the circulating numbers of these cells remain stable following exercise.²⁷

There are three likely mechanisms to explain the exercise-induced leukocytosis. The first mechanism involves hemoconcentration resulting from shifts in fluid balance with the overall effect of decreasing the extracellular fluid compartment.^{102,114,115} The increases in the packed cell volume and total protein concentration due to intense exercise are partially due

to this mechanism as well.^{116,117} The second mechanism involves catecholamine levels, which have been shown to rise in response to exercise and are known to mobilize leukocytes from the marginal pool.⁹⁵ The third mechanism that may play a role in the leukocytosis of exercise involves the mobilization of granulocytes from the marrow pool caused by exercise-induced increases in cortisol concentration.^{102,118–120} It is believed that this latter mechanism is responsible for the previously mentioned biphasic nature of exercise-induced leukocytosis.^{77,93,95,99,120,121}

Exercise and leukocyte function

Single bouts of moderate to intensive exercise are associated with an initial increase in NK or LAK cell function in humans^{26,28,107,122} and horses²⁷ but severe exercise is followed by depression of NK function.¹²² Single bouts of moderate to intensive exercise transiently impair neutrophil antimicrobial functions in both horse and human.^{21,98} High-intensity exercise on a treadmill has been associated with a general reduction in neutrophil function in both species.^{44,123} Endurance racing is likewise associated with negative changes in neutrophil function in both man and horse.^{46,47} Similar effects of intensive exercise on bronchoalveolar macrophage function are also seen in horses,^{23,24} although in mice there appears to be an enhancement of macrophage function following an exercise challenge.²² As such, these studies indicate that equine innate immune functions, like those of humans, can be adversely affected by intensive exercise.

There are relatively few reports of exercise-induced changes in antigen-specific lymphoproliferation as the majority of the work on exercise-induced changes in lymphocyte function has concentrated on the mitogen-specific response. In general, intense exercise leads to decreased lymphoproliferation to both mitogens and antigens.^{11,12,124} The magnitude of this effect has been shown to grow with increases in exercise intensity and duration.^{36,38,39} In one study in horses, there was no change in the lymphoproliferative response to the various mitogens.²⁴ In contrast, other studies have shown that unconditioned horses exhibit decreased lymphoproliferative responses to both mitogens and equine influenza virus in response to a single bout^{43,44} and multiple bouts of intensive exercise.³⁰ Studies involving conditioned horses found decreased lymphoproliferative responses to mitogens in response to

Table 7.2.2 Basal immune responses of trained and untrained horses⁴³

Immune response	Untrained horses	Trained horses
Mitogen proliferation	68 266 ± 2082 cpm	68 066 ± 5428 cpm
Influenza proliferation	7358 ± 426 cpm	8480 ± 1494 cpm
LAK cytotoxicity	16.2 ± 1.8 lytic units	12.8 ± 3.1 lytic units

LAK, lymphokine-activated killer.

treadmill-based exercise challenge⁴⁵ and racing.²⁹ While positive training effects have been observed on human basal immune responses,^{4,17} exercise-conditioned horses do not appear to have a similar increase in their basal immune responses (Table 7.2.2).

More recently it has been suggested that strenuous exercise might also alter patterns of cytokine gene expression, though the absolute effect varied.^{32,33,125} Production of the pro-inflammatory cytokines interleukin (IL)-1, IL-6, IL-8, IL-12, granulocyte/macrophage-colony stimulating factor (GM-CSF), and tumor necrosis factor (TNF)- α is enhanced by intensive exercise,^{126–128} suggesting that exercise is a form of limited inflammatory response.¹²⁹ IL-6 is thought to play a central role in this process as it is produced in large quantities in response to exercise and locally in skeletal muscle in response to exercise.¹³⁰ From these results, it has thus been proposed that skeletal muscle damage during physical exercise results in an inflammatory response.^{131,132} It has recently been reported that multiple bouts of high-intensity exercise may be more potent inducers of this inflammatory response.^{133,134} Although the overall significance of these results remains controversial,¹³⁵ it is becoming increasingly apparent that exercise is associated with a pro-inflammatory cytokine response. Whereas these cytokines themselves have immunoregulatory functions, their production may also have a role in post-exercise immune suppression.^{136,137} The theory is that the counter-regulation of the initial inflammatory immune response involves the production of cortisol, prostaglandins, and IL-10 (an anti-inflammatory cytokine), all of which contribute to the post-exercise immune suppression.^{130,137} This mechanism is also consistent with reports that exercise may enhance Th2 immune responses at the expense of Th1 responses.³² IL-10 and corticosteroids suppress Th1 responses and favor Th2 development.¹³⁷ Intensive exercise-induced inhibition of Th1 cytokine production also occurs in horses,³⁰ although the role of IL-10 in this process is unknown. Clearly the effect of exer-

cise on cytokine production in the horse needs further work.

The effects of exercise on humoral immunity are conflicting at best. Prolonged periods of intense training lead to an impairment of mucosal immunoglobulin levels in humans.^{2,138–140} Exercise appears to have little effect on immunoglobulin concentrations or antibody production in horses.^{30,98} Actively exercising horses and humans produce similar levels of antibodies following vaccination as unexercised controls;^{34,141} however, cell-mediated immunity following vaccination may be impaired.^{30,142,143} The possibility of altered patterns of immunoglobulin isotype production in response to exercise has not been addressed, although such alterations have been described in other stress models.^{86,87} Additional work needs to be done to elucidate the effect of exercise on immunoglobulin levels and isotype production in both horse and man.

Mechanisms of exercise-induced changes in immune function

The mechanisms responsible for changes in immune function are unknown, although much of the data seem to implicate a central role for cortisol and/or catecholamines. Exercise has been shown to increase the plasma concentration of cortisol and catecholamines significantly and the magnitude of this response is directly related to the intensity and duration of exercise.^{54,77} Hormonal responses of the horse to exercise have been shown to mimic the response of humans and other mammals in which both cortisol and catecholamine levels have been shown to increase significantly in response to high-intensity exercise.^{68,100,144–146} Again, the general consensus is that cortisol mediates the later changes following exercise while the early or immediate effects of exercise are due to the catecholamines (Fig. 7.2.3).

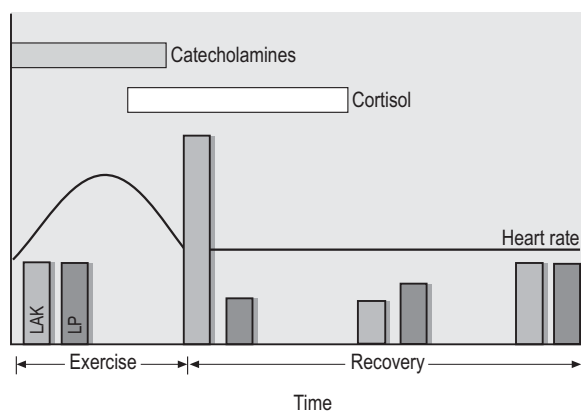


Fig. 7.2.3

Equine exercise-immunology model. Exercise is associated with the early production of catecholamines and an increase in heart rate. Cortisol production occurs later and is intensity-dependent. Lymphokine-activated killer (LAK) cell activity and the lymphoproliferative (LP) response to mitogens are at a baseline level prior to exercise. Upon completion of exercise, LAK activity increases with a concomitant decrease in the LP response. Later in the recovery period, both LAK and LP responses are suppressed, although eventually they will return to baseline levels. The magnitude and duration of the effect on LAK and LP is also intensity-dependent.

Production of cortisol, the primary secretory glucocorticoid, in the horse has been well studied but the actual physiological significance of the changes that occur following exercise is far from being understood. Plasma cortisol in the horse has a half-life of 70–100 min and has been shown to peak 20–30 min after the cessation of exercise.¹⁴⁵ It has been determined that plasma cortisol levels do not increase due to exercise until the workload reaches the 60% maximum oxygen uptake ($\dot{V}O_{2\text{ max}}$).^{30,147–149} The increase in plasma cortisol concentration is the result of a change in the balance between the combined increased secretion of cortisol and increased clearance rate in response to exercise, with the secretion rate showing the greater increase.⁶⁷ Exercise training is associated with an adaptation of the HPA axis leading to decreased pituitary sensitivity to corticosteroids and increased plasma cortisol levels.^{150,151} Exercise-induced alterations in glucocorticoid receptor expression and signaling in lymphocytes have also been reported in human athletes,^{152,153} and this can lead to decreased sensitivity of corticosteroid suppression of immune function.¹⁵⁴ No similar information is available for the horse.

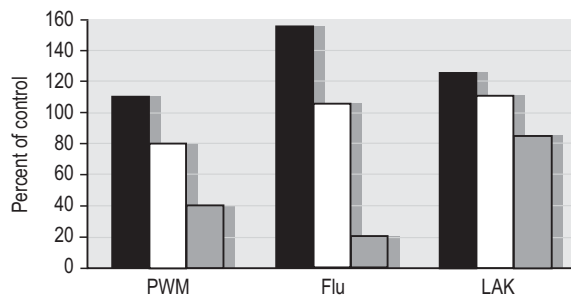
Because corticosteroids are known to be potent anti-inflammatory agents and to have dramatic effects on immune cell function,⁶⁹ it is tempting to attribute exercise-induced changes in immune function to increases in plasma cortisol. Thus corticosteroids inhibit synthesis of various cytokines such as IL-1, IL-2, IL-3, IL-6, interferon gamma (IFN- γ), GM-CSF, and TNF- α .^{69,155,156} Likewise, the production or release of lymphotoxin, monocyte chemotactic factors, vasoactive amines, prostaglandins, and plasminogen activator is diminished in the presence of corticosteroids.^{69,155,156} Indirect corticosteroid effects on *in vitro* lymphoid cell function have likewise been reported, presumably resulting from altered regulation of cytokine production and activity and/or antigen-presenting cell function.¹⁵⁷ Thus a variety of research has demonstrated corticosteroid-related decreases in mitogen responses, LAK cell activity, CTL function, NK cell-mediated cytotoxicity, and lymphocyte proliferative responses to specific antigens.^{54,158–160} Although the magnitude of the cellular response to glucocorticoid depends upon the hormone level to which it is exposed, the relationship between corticosteroids and the immune system is complex and may be obscured by factors such as steroid preparation and species sensitivity to corticosteroids.¹⁶¹ Studies that demonstrate corticosteroid-associated immune suppression *in vitro* often utilize synthetic preparations and/or non-physiologic concentrations of the steroid. Synthetic hormones such as dexamethasone or prednisolone have a longer half-life in circulation, bind less strongly to cortisol-binding globulin, and have a higher affinity for the corticosteroid receptor and are thus more potent than cortisol.^{162,163} Pharmacologic concentrations of cortisol that are immunosuppressive are not relevant to the *in vivo* situation.¹⁶³ Finally, it should be noted that most studies on the effects of corticosteroids have been performed on corticosteroid-sensitive species such as rats and mice.¹⁶⁴ In these animals, relatively small amounts of corticosteroids have dramatic suppressive effects on immune function, including thymic involution and lymphoid cell death.¹⁶⁴ In corticosteroid-resistant species such as the horse, immune suppression by corticosteroids may be absent or else require even higher doses and prolonged exposure to corticosteroids for expression.¹⁶⁵ It is important to keep these caveats in mind when considering the role that cortisol may play in exercise-induced immune modulation. To address some of these issues we have tested equine peripheral blood mononuclear cells *in vitro* for the effect of cortisol on lymphoproliferative and LAK cell function (Fig. 7.2.4). Significant effects

Table 7.2.3 Hematologic results from an intravenous injection of cortisol or vehicle⁴³

	Cortisol			Vehicle		
	−60	+20	+120	−60	+20	+120
WBC	8.4	7.7*	8.5	10.9	11.0	11.2
Neuts	5.1	4.9*	6.0	6.8	6.6	6.7
Lymphs	2.8	2.5	2.0*	3.0	3.2	2.8
N/L	1.8	2.0	3.0*	2.3	2.1	2.4
LAK	62.3	53.2	108.9	52.9	58.2	58.5
PWM	35.3	35.0	33.4	28.4	31.5	35.7
Flu	4.3	1.9	3.6	3.4	2.3	4.1

Flu, proliferative response to influenza virus ($\times 10^3$ net cpm); LAK, lymphokine-activated killer — cell activity as lytic units per 10^6 cells; lymphs, lymphocytes $\times 10^3/\text{mL}$; neuts, neutrophils; N/L, neutrophil/lymphocyte; PWM, proliferative response to pokeweed mitogen ($\times 10^3$ net cpm); WBC, white blood cells.

*Significantly different from −60 timepoint ($P < 0.01$, RMANOVA).

**Fig. 7.2.4**

The effect of cortisol on equine proliferation and lymphokine-activated killer (LAK) cell activity. Peripheral blood mononuclear cells were incubated with increasing concentrations of cortisol (10^{-10} , 10^{-8} , and 10^{-6} M, black, white and gray bars, respectively) and either equine influenza virus antigens (Flu) or pokeweed mitogen (PWM) and the proliferative response determined by ^3H -thymidine incorporation on days 5 and 3, respectively. Cortisol was added to equine LAK cell cultures at the same doses and cytotoxic activity assayed as previously described (see Table 7.2.3). Data are presented as the average $\pm$ SE of the percentage of the response of cultures not receiving any cortisol.

were only observed at the highest (10^{-6} M) concentrations. Likewise, injection of a single bolus of cortisol at physiologic concentrations failed to alter immune cell function, although hematologic responses were observed (Table 7.2.3). Together, these results indicate that cortisol alone may not account for exercise-induced changes in immune cell function in the horse.

Catecholamines have also been shown to increase significantly in response to exercise in the horse.^{146,166} Epinephrine (adrenaline) and norepinephrine (noradrenaline) are the major secretory catecholamines and have similar functions including enhancement of cardiac contraction, blood flow to the heart and skeletal muscle, blood glucose, lipolysis, and oxygen consumption.^{57,72} The plasma half-life of both epinephrine (adrenaline) and norepinephrine (noradrenaline) is less than 30 s in the horse.¹⁶⁶ As is the case for cortisol, catecholamine secretion has been shown to increase as the intensity of exercise increases.^{166–168}

Catecholamines exert some of their effects on immune responses indirectly by causing the redistribution of the blood cells leading to leukocytosis, lymphocytosis, neutrophilia, decreases in the CD4:CD8 ratio, and increases in NK cell numbers.^{25,75,95,169} Catecholamines also directly influence antigen-specific antibody responses, mitogen-stimulated lymphocyte blastogenesis, and natural cytotoxicity.^{170–172}

In addition to the effects of cortisol and catecholamines on the immune system, several other mediators have been implicated as having a potential role in the changes seen following exercise. These include β -endorphin, growth hormone, prolactin, and enkephalin.^{126,149,173,174} Among these, β -endorphin has been the most extensively studied in terms of its effect on immune cell function. β -Endorphin release has been shown to increase following intense exercise requiring intensity in excess of 60% $\dot{V}\text{O}_{2\text{ max}}$.^{149,173,174} β -Endorphin has been shown to be generally immunosuppressive.^{54,126}

Exercise and infectious disease

The central question remains, does exercise lead to increased susceptibility of horses to bacterial and viral infections? Although the physiologic and immunologic consequences of intensive exercise parallel the effect of other physical and psychological stressors,^{53,54} do these changes in immunologic function measured in vitro correlate with increased susceptibility to disease in vivo? Epidemiologic surveys have confirmed an increased incidence of upper respiratory tract infection symptoms in human athletes following participation in high-intensity events, such as marathon races,^{89–91} although the precise mechanism responsible for this increase remains uncertain.^{175–177}

The marked susceptibility of race horses to respiratory infections is widely recognized.^{178–180} The reasons for this remain unknown, although they are likely to include the immune status of the susceptible population as well as exposure to infected individuals.^{181,182} Interestingly, a recent history of vaccination is not necessarily associated with a reduction in disease risk.¹⁸² The possibility of training or racing schedules affecting the incidence of disease has not been widely considered.¹⁸¹ In a recent study using a treadmill-based exercise protocol it was shown that vaccinated ponies subjected to repeated bouts of high-intensity exercise were susceptible to influenza virus infection, whereas non-exercised controls were protected.³⁰ This increased susceptibility was associated with alterations in the in vitro cell-mediated immune response to the virus.³⁰ Could a similar exercise (stress)-induced alteration in immune function account for influenza infections in vaccinated race horses? Do exercise-induced alterations in neutrophil function likewise leave endurance and race horses susceptible to bacterial infections? Although there is little information currently available to support these concerns, the

experience from human sports medicine does suggest that there could be a similar 'window of vulnerability' in our equine athletes.

Assuming that there is an association between exercise-induced immune suppression and increased incidence of respiratory disease in the horse, what can be done? Some attempts have been made through chemical or nutritional means (e.g. indometacin, glutamine, vitamin C, and carbohydrate supplementation) to attenuate immune changes after intensive exercise,^{9,183,184} although no consistent relationship between nutritional interventions, exercise immunology, and upper respiratory tract infection risk has yet been established.¹⁸³ Increased carbohydrate ingestion was associated with an attenuated cortisol, growth hormone, and epinephrine (adrenaline) response and fewer perturbations in immune function.⁹ Whereas dietary manipulation has been widely recognized as a means to meet the energetic needs of the equine athlete,^{185,186} the possible effect of diet on immunocompetency in the exercising horse has not yet been addressed.

Conclusions

Our understanding of the effect of exercise on the immune system is improving, although a number of issues remain unresolved, including the in vivo significance of some of the in vitro findings. Further work defining the mechanisms and long-term consequences of exercise-induced immune modulation is needed before specific changes in current training or vaccination programs can be contemplated. Nevertheless, evidence from the human literature indicates that such efforts could prove useful in protecting the health and welfare of the equine athlete.

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Effects of exercise on gastrointestinal function

Mireia Lorenzo-Figueras & Alfred M. Merritt

Background

Based upon the design of its gastrointestinal tract (GIT) alone, the horse would be an unlikely animal for humans to choose as their athletic alter ego. The very large cecum and folded large colon, normally filled with ingesta, must certainly not be the easiest structures to move around. Likewise, the anatomical arrangement and complexity of function of these fermentation vats could, theoretically at least, set the system up for any number of exercise-induced abnormalities. When left to their own devices in the wild, herbivores, whether they be the forestomach fermenters or the hindgut fermenters which include the equidae, spend the large majority of their day quietly grazing or resting.¹ Instances of movement faster than a walk are usually brief and in response to attack by a predator or a rival.

'Domesticated' horses, on the other hand, are often subject to many situations that result in a greater percentage of their level of daily activity being much more vigorous than just walking and standing around grazing. While hard data concerning the effect of any form of exertion on the equine GIT, whether it be direct or indirect, are scarce, we have some results showing that even the anticipation of an upcoming training session on a treadmill, such as turning on the treadmill motor, will evoke an increase in intra-abdominal pressure, reflecting tensing of the abdominal muscles.² One outstanding effect this pressure increase, and that associated with subsequent trotting or galloping, has on the stomach, for example, will be discussed later in this chapter. The point is that the intra-abdominal pressure response could have far-reaching effects on abdominal viscera, and that the character of the response could vary depending upon the type and duration of the exertion. Galloping may have a different effect than trotting, while trotting

may have a different effect than pacing. Jumping may have entirely unique consequences. Effects could include distortion and/or displacement of viscera, redirection of blood flow, and release of various regulatory peptides.

A study of human subjects that were 'encouraged to perform at near-maximal effort' indicated that runners have more complaints of gastro-esophageal reflux (GERD) than do cyclists.³ Could this be because, in the bent-over position in which they ride, cyclists create less abdominal muscle press than do runners? Or is it because, as one report has suggested, the physical displacement of the abdominal viscera associated with the 'pounding of the pavement' results in an irritation that produces symptoms — the so-called 'cecal slap syndrome'?⁴ Or, could it be a question of experimental methodology, since two subsequent studies found that subjects asked to perform at up to 90% $\dot{V}O_{2\max}$ on a stationary bicycle experienced GERD that was exercise intensity-dependent?^{5,6} That is, type, duration, and intensity of exercise all need to be considered from the standpoint of potentially differential effects on the gut.

Finally, considering all of the things humans ask horses to do in the form of exertion, are some forms more stressful than others? In general, existent data indicate a direct relationship between plasma adrenocorticotrophic hormone (ACTH) and cortisol concentrations and intensity and duration of exercise, respectively, in horses.⁷ But in one study, for instance, galloping caused no significant increase in pituitary venous blood corticotrophin-releasing hormone (CRH), although ACTH concentration did increase significantly.⁸ These issues must also be factored in with respect to the potential impact on the GIT.

This chapter considers the potential effects of exercise on:

- digestion
- absorption
- secretion
- motility
- maintenance of mucosal barrier integrity
- liver-specific functions.

These functions are directly influenced by blood flow status, and activity within the enteric nervous system (ENS), which may be strictly intrinsic or may be modified by input from the central nervous system (CNS). With respect to horses, some special species-relevant aspects of these functions deserve note.

Digestion

Some fermentation of soluble carbohydrate occurs within the stomach, with the production of lactic and volatile fatty acids (VFAs). Whether and to what extent these products are absorbed across the gastric wall and into the bloodstream is not known, although a large proportion should be in the non-ionized, diffusible form, given the acidic environment. Pancreatic secretion, on the other hand, while being large in volume, is very low in amylase and protease activity. The usual complement of small intestinal (brush border) disaccharide enzymes is present. However, small intestinal proteolytic enzymes have not been measured in horses. From a comparative perspective, the one thing that makes horses unique with respect to other domestic species is the degree of cecal and colonic digestion of dietary fiber; up to 35% of the animal's daily caloric needs may be derived from the volatile fatty acids produced from the fermentation of dietary fiber.⁹

Absorption

As indicated above, one of the most unique aspects of equine gut function is the fermentation of carbohydrates at both ends of the tract. While we still know little about where and how the products of gastric fermentation are absorbed, we do know that absorption of VFAs produced in the cecum and colon requires mucosal Na^+/H^+ exchange. A mole of water follows each mole of VFA to be absorbed. It follows that disruption of any of the mechanisms involved in support of normal mucosal function — because of an exercise-related event, for example — could have major consequences on water and electrolyte balance, and on nutrition. In addition, under conditions where the cecum and colons are presented with a large amount

of fermentable carbohydrate over a short period of time, the production rate of the VFAs may initially outstrip absorption and, through the osmotic gradient created, pull water from plasma into the colonic lumen.

Secretion

Gastric acid secretion occurs in the horse even under fasting conditions.¹⁰ The important role played by acid in gastric squamous mucosal ulcerogenesis has been demonstrated, especially as related to the high incidence of this form of gastric ulcer disease in horses under intensive training conditions.^{2,11–13} This association probably provides one of the clearest representations to date of how exertion can affect the equine GIT (see below). As mentioned above, from a comparative perspective, pancreatic secretion in horses is very high in daily volume — estimated at 50–60 L/day — but is relatively low in bicarbonate and amylase and protease enzyme concentration.¹⁴ At least when the stomach is empty, large volumes of small intestinal contents, made up primarily of pancreatic juice, periodically reflux into the stomach, providing some buffering of the acid present.¹⁵ The effect of exercise, if any, on this process still needs to be investigated. Finally, the horse undoubtedly possesses all the intestinal secretory mechanisms that have been described in other species, but to what degree needs to be defined. What must be remembered is that, in contrast to humans and animals with relatively simple colons, a hypersecretion of strictly small intestinal origin in the horse would not manifest as diarrhea because of the ability, from a water balance perspective, of the cecocolon to compensate for that malfunction.¹⁶ Thus, diarrhea in adult horses is indicative of a large bowel dysfunction, which could include hypersecretion at this level.

Motility

As an extension of the human experience,¹⁷ horse trainers often try to schedule the training sessions for a time when they think the horse has a relatively empty stomach. Humans in general do not feel that they can perform maximally on a 'full stomach.' It has been shown that pedaling exercise slows gastric emptying rate in humans, although more runners than cyclists complain of GI problems.^{18–20} The effects of exercise on gastric motility and emptying function in horses are not known and require study, as they may have important implications regarding squamous

mucosal ulcerogenesis. As in other species, the proximal stomach relaxes in response to active ingestion (receptive relaxation), and the degree of this relaxation is directly related to the amount of feed ingested.²¹ A more sustained, but less profound, post-ingestion proximal relaxation (accommodation) then occurs, along with peristaltic-like contractions that begin in the middle of the gastric body and traverse through the antrum and promote emptying of gastric contents into the duodenum. The presence of those contents within the duodenum will, in turn, have modulatory effects on the rate of gastric emptying, the degree of which will depend upon their specific composition.²² This complex regulatory process could be affected by exertion. As indicated earlier, intragastric fermentation of ingested soluble carbohydrates occurs to a considerable degree in horses, and this implies gas production as a result. It follows that this gas must also be moved aborally into the duodenum, since horses do not normally eructate. Likewise, the large amount of gas that is generated from hindgut fermentation must be moved aborally, along with the contents, to be expelled via the anus. Thus, any condition that could adversely reduce the delivery of gas and/or contents, such as an exercise-induced dysmotility, could result in notable abdominal discomfort and/or diarrhea.

Maintenance of mucosal barrier integrity

Normal gut function is dependent upon a reasonably intact barrier between luminal contents and the sub-mucosa.^{23–25} Maintenance of barrier integrity is dependent upon numerous factors that include blood flow, mucosal cell turnover rate, and immunological tolerance to luminal antigens. Methods for experimentally evaluating the ‘leakiness’ of GI mucosa are discussed below. From the few *in vivo* and *in vitro* studies done to date, it appears that horses probably will not differ fundamentally from other species with respect to regional mucosal barrier characteristics, although this needs much more investigation.^{26–31}

Liver-specific functions

Irrespective of species, normal liver function is critical to good health, and this is highly dependent upon adequate blood supply from both arterial and portal venous sources. Blood supply is emphasized here because it is probably the aspect of liver function most prone to modification by exercise. From a species-specific perspective, it should also be kept in mind that

certain detoxifying and conjugative functions of the equine liver might be prone to modulation by fasting and fever, as is the case for endogenously generated bilirubin.³²

Available methodologies to document the effects of exercise on the equine gastrointestinal system

Methods that have been used in horses

Transit markers

Gastrointestinal transit time can be measured by the use of indigestible, non-absorbable markers. The rate of passage is normally expressed as mean retention time (amount of marker excreted at a certain time after administration of the marker), cumulative excretion or % recovery rate.

Comparison of rate of digesta passage during rest and light to moderate exercise has been evaluated in horses and donkeys. Some of the markers used in these studies were cobalt-EDTA, for liquid phase, chromium-mordanted fiber, for solid phase, and ruthenium-phenanthroline and ytterbium chloride, for particulates passage.^{33–35} Non-absorbable markers, such as phenol red in human^{18,36,37} and horse³⁸, and polyethylene glycol in dog³⁹, have been used to measure the effect of different levels of exercise on gastric emptying (GE) of liquid meals. After ingestion of a labeled meal, gastric contents are aspirated through a nasogastric tube or recovered by drainage through a gastric cannula. The amount of marker remaining in the stomach after exercise will depend on the rate of GE of the labeled meal. However, repeated nasogastric intubation *per se* may affect gastric emptying rate.

Apparent digestibility of diet

Changes in efficiency of dietary fiber utilization in response to exercise may reflect changes in the passage rate of digesta, the activity or population of microbes within the gut, or mucosal integrity.⁴⁰ Apparent digestibility is calculated as the difference between specific components of the diet and those found in feces, expressed as percent utilization. The effect of exercise on diet digestibility has been studied in horses.^{33–35}

Barostat

The electronic barostat has been extensively used to document gastrointestinal motility in many species, including horses.^{2,41–43} Unlike other methods, this system is more useful for recording changes in smooth muscle tone than active, phasic contractions. The principle of the barostat is to maintain a constant pressure within a plastic bag of infinite compliance, positioned within the lumen of the segment to be studied. When the internal pressure of the organ increases for any reason (for example, contraction, increased mural tone), the barostat aspirates air from the bag to maintain the intrabag pressure constant. Conversely, when the internal pressure decreases, air is injected into the bag. Therefore, as the bag follows the movement of the visceral walls, changes in bag volume are an indirect measurement of changes in tone of the organ (Fig. 8.1.1).⁴⁴

In other instances, changes in bag volume reflect changes in external pressure exerted over the organ containing the bag. As we mentioned earlier, we

observed such changes in the proximal stomach of horses under a training session² and related them to increased intra-abdominal pressure caused by tensing of the abdominal muscles during exercise. Interestingly, in some horses we also observed relaxation of the proximal stomach soon after exercise (data not published). What effect this might have on gastric emptying, for instance, could provide some useful information concerning optimal timing of feeding with respect to a given exercise schedule.

Blood flow measurement

Adequate local perfusion is necessary for normal gut tissue activity, which requires oxygen and nutrients supplied by peripheral blood. Exercise leads to blood flow diversion from the gastrointestinal tract to the working skeletal muscles and the skin.⁴⁵ Decreased perfusion or ischemic damage may result in impairment of normal function of the gastrointestinal tissues, including mechanisms of mucosal protection, mem-

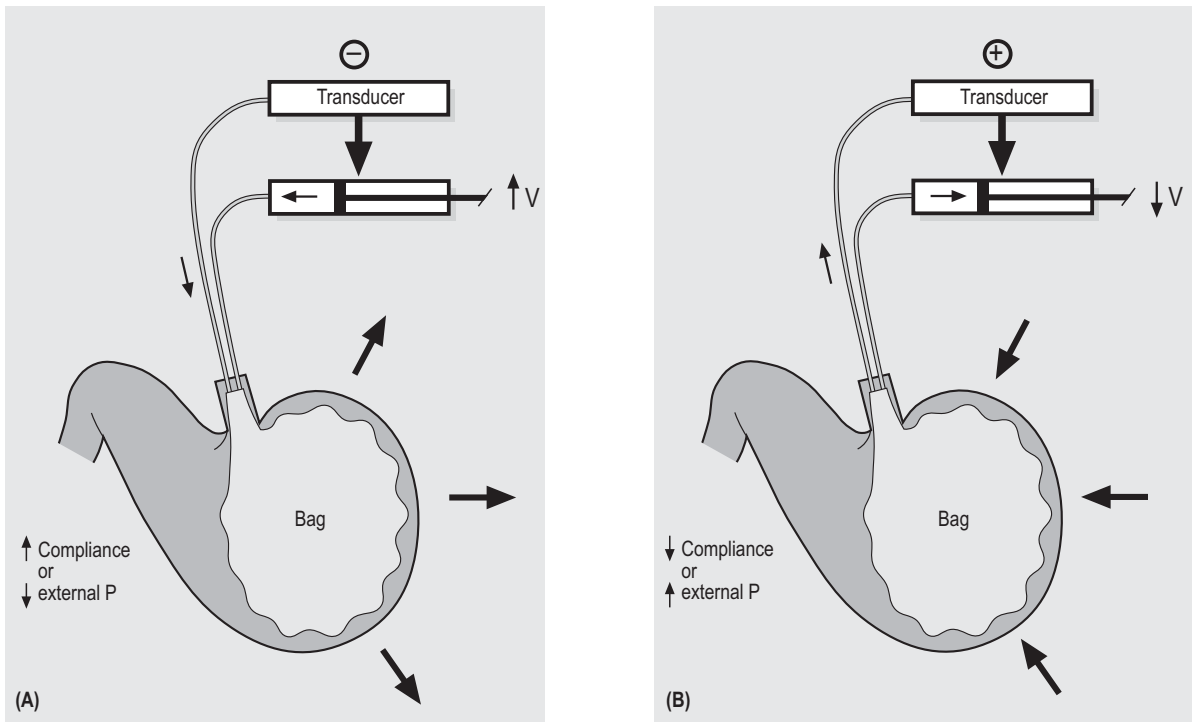


Fig. 8.1.1

Motility of the stomach can be indirectly measured by an electronic barostat. The barostat maintains a constant pressure (P) within an intragastric bag. (A) When the stomach relaxes or the gastric wall becomes more compliant, the system injects air into the bag. (B) Conversely, when the stomach contracts or the gastric wall becomes less compliant, air is aspirated from the bag. Changes in bag volume (V) can also reflect changes in pressure exerted extraluminally upon the stomach. The same method could be applied to study motility at any site of the intestine.

brane secretory and absorptive functions, and motility.

The effect of short-term exercise on blood flow of abdominal organs has been measured in the horse, using radionuclide-labeled microspheres.⁴⁶ Once injected into the general circulation, the distribution of microspheres is proportional to the blood flow during its first transit through the circulation.^{47,48} Consequent quantification of radioactivity within tissue preparations from horses that have been exercised indicates the level of regional blood flow to those tissues.

Additionally, hepatic blood flow can be quantified by intravenous infusion of a marker, such as bromsulphalein (BSP). As BSP is highly extracted by the liver, hepatic clearance of BSP is mainly dependent on liver blood flow. Decreases in blood clearance of BSP have been attributed to exercise-induced splanchnic vasoconstriction, leading to decreased portal vein blood flow.⁴⁹

Continuous pH monitoring

Continuous pH monitoring of the GIT luminal contents with a self-referencing pH electrode may be useful for tracking the effects of exercise on digestive function. The stomach and distal small colon are accessible without special preparation; other parts of the tract would need to be surgically cannulated. Continuous recording of pH changes within the proximal portion of the stomach has suggested that exposure of the squamous mucosa to hydrochloric acid is increased during exercise (Fig. 8.1.2).² There are no data in the literature that describe pH changes within the small colon in response to exercise. Such data have the potential to provide information about the effect of exercise on mucosal secretion, net electrolyte flux, and fermentation activity within the large intestine.

Measurement of GI regulatory peptides and steroids

Modification of gastrointestinal regulatory peptides and steroids in response to exercise may either result in, or reflect changes in, gastrointestinal function. Some of these substances have been measured in horses under different exercise regimens, varying in type, intensity, and duration.

Exercise stimulates adrenal secretion and activity of the sympathetic autonomic nervous system. This stimulation is reflected in increased plasma levels of ACTH, catecholamines, cortisol, and β -endorphins,^{7,50–52}

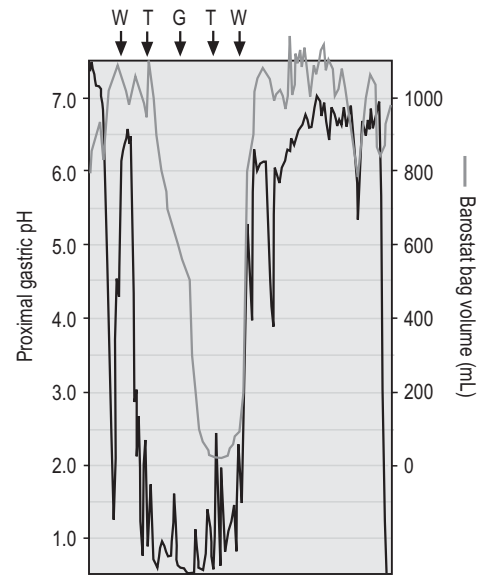


Fig. 8.1.2

Composite of continuous pH and intragastric barostat bag volume monitoring in two different horses respectively during exercise. In gray: changes in intragastric pressure were indirectly measured by changes in volume of a bag placed within the proximal stomach, with its internal pressure constantly controlled at 6 mmHg by an electronic barostat. The decrease in volume indicates increased pressure >6 mmHg on the bag. In black: intragastric pH was measured by a self-referencing pH electrode positioned 2 cm below the entrance of the stomach. Both patterns are characteristic responses to exercise. W, walk; T, trot; G, gallop.

which are used as indices of physical stress. As the horse's fitness improves, these hormonal responses to exercise diminish.⁵³ Variations in plasma cortisol are more sensitive to duration of exercise, whereas ACTH and catecholamine levels are more closely correlated with exercise intensity.^{7,54} The effect of variations of stress hormone levels upon equine gastrointestinal function is still unknown. In other species, cortisol can have a deleterious effect on mucosal immune function and epithelial permeability,⁵⁵ whereas catecholamines have the potential to delay gastric emptying, prolong transit time,^{56,57} and decrease perfusion of the gastric mucosa.⁵⁸

Gastrin is a major regulatory peptide of acid secretion, the plasma concentration of which was increased after prolonged exercise in the horse in one study,⁵⁹ but not in another.⁶⁰ In a third study, gastrin concentration did not increase after short, sprint exercise, but

was higher post-prandially in trained horses, compared to non-trained horses.⁶¹ Plasma concentrations of inhibitors of gastric acid secretion, such as somatostatin in man⁶² and vasoactive intestinal polypeptide (VIP) in man⁶³ and horse,⁵⁹ may increase with exercise. Additionally, somatostatin may favor colonic electrolyte transport and inhibit nutrient absorption.⁶⁴

Potential exercise-induced changes in fluid absorption and secretion within the gut may reflect changes in expression of some regulatory hormones, and vice versa. Exercise increases plasma levels of secretagogues, such as VIP and glucagon, in the horse,⁵⁹ and secretin, gastric inhibitory polypeptide, and prostaglandins in man.^{62,65,66} On the other hand, atrial natriuretic peptide and aldosterone, which favor sodium absorption, also increase during prolonged exercise in horses.^{51,67}

Agents such as motilin,⁶⁸ neuropeptide Y,⁶⁹ and prostaglandins⁶⁶ can affect motility and are released during endurance-type exercise in man. The effect of exercise on GI motility can, therefore, be indirectly studied by measuring endogenous expression of hormones that control intestinal motility.

Finally, alterations of hormone levels may result from a decrease in GIT blood flow, leading to decreased hepatic and renal clearance.⁴⁵ VIP⁵⁹ and arginine vasopressin⁸ in horses, and endothelin-1⁷⁰ and angiotensin II⁷¹ in other species, are released during exercise and act as vasoconstrictors. The resulting decrease in blood flow may affect function and regulation of the GIT.

Methods that could potentially be useful in horses, based upon experience in other species

Gastric motility and emptying

In contrast to the phenol red technique mentioned above, barium *contrast radiography*⁷² and *scintigraphy*⁷³ have the advantage of non-invasiveness, and have been previously applied to equine studies. An additional advantage of the latter technique is the differentiation between solid and liquid-phase emptying.

Breath tests employing stable isotopically labeled tracers are potentially very useful, since they are non-invasive, non-radioactive, and easy to perform (Fig. 8.1.3). As it empties the stomach, the ¹³C-enriched marker is readily absorbed in the proximal intestine

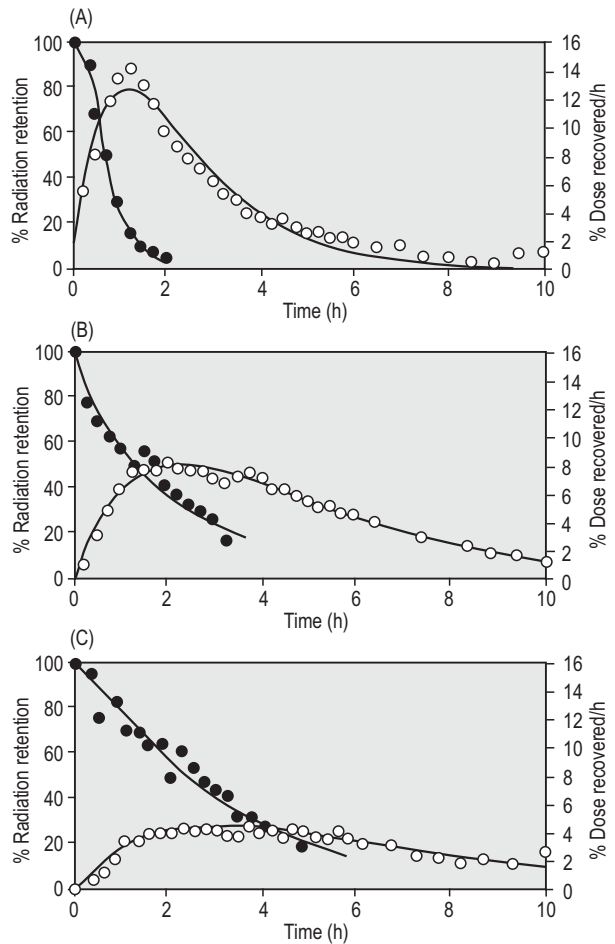


Fig. 8.1.3

Results of simultaneous ¹³C-octanoic acid breath test and gastric radioscintigraphy in three typical cases. (A) Rapid gastric emptying rate ($T_{1/2} = 0.73$ h). (B) Normal gastric emptying curve ($T_{1/2} = 1.34$ h). (C) Slow gastric emptying pattern ($T_{1/2} = 2.62$ h). Scintigraphic data (●) on the left y-axis and breath test data (○) on the right y-axis are plotted against time. The continuous lines represent a modeled fit. (Reproduced with kind permission from Sutton et al.⁷⁶)

and metabolized to CO₂. Thus, breath ¹³CO₂ enrichment reflects the rate of GE of the labeled meal. The effect of exercise on GE of carbohydrate meals marked with ¹³C-acetate has been studied in humans.^{74,75} The ¹³C-octanoate breath test has been validated in the horse⁷⁶ and its use in exertional studies is promising.

Imaging of the stomach by ultrasound has shown that strenuous exercise disrupts gastric motility in humans, although the examination was done right after, rather than during, the active exertion.⁷⁷

Another available technique in humans is electrogastrography (EGG), which consists of measuring gastric myoelectrical activity by electrodes positioned on the surface of the epigastrium.⁷⁸ Although both techniques as applied to the stomach are unsuitable in the horse for anatomical reasons, they could be potentially used to measure cecal motility percutaneously — also known as electrocectography⁷⁹ — during the recovery period after exercise.

Gastric secretion

Collection of gastric contents from cannulated dogs has allowed constant measurement of acid and pepsin during exercise.³⁹ This method could be useful in determining changes in acid secretion and other gastric components in cannulated horses. It would require that the animal be fasted, however.

Gastric ischemia

Air tonometry measures changes in luminal intragastric PCO_2 and seems to be a more reliable indicator of gastric ischemia than measuring splanchnic blood flow. Detection of ischemia is based on the difference between arterial PCO_2 and intragastric PCO_2 , which better reflects mucosal balance between needed and supplied levels of O_2 .⁸⁰ It would be interesting to apply this technique to the horse.

Intestinal transit and motility

Mouth-to-cecum transit time can be measured by the H_2 breath test during exercise.^{81,82} For this test, lactulose, a non-absorbable disaccharide, is added to the meal. Breath H_2 starts rising when lactulose reaches the cecum and is degraded by bacterial fermentation. Although fermenting bacteria are also present in the equine stomach and small intestine, the cecum and the large colon are the primary sites of microbial fermentation.⁸³ However, not all horses exhale excess hydrogen after ingestion of lactulose, so this technique may not be reliable.⁸⁴ Lactose, another disaccharide, has been used as a marker for the H_2 breath test,⁸⁴ since adult horses appeared to be lactose-intolerant.⁸⁵ Yet the presence of brush border lactase in the small intestine of adult horses has been recently reported,⁸⁶ and also excludes the usefulness of this sugar to measure oro-cecal transit time. The lactose- ^{13}C ureide breath test (LUBT) is another method under investigation in horses. The labeled lactose ureide reaches the large intestine intact, where it is hydrolyzed by bacte-

ria, producing ^{13}C -labeled CO_2 . In contrast to the H_2 breath test, LUBT seems to be a repeatable and more reliable method.⁸⁷

Motor activity of the intestinal tract can be studied by several invasive techniques. For example, *electrodes* implanted on the serosa of the gut can detect surface potentials or voltages generated by the gastrointestinal muscle. These electrodes measure two different patterns of electrical potentials: *slow waves*, which reflect basal electric rhythm, and *action potentials*, which correlate with contractions. By implantation of bipolar electrodes in dogs, slow wave patterns and the migrating motor complex (MMC) of the proximal jejunum have been studied in relation to exercise of different intensities.⁸⁸ This technique has certainly been successfully applied to horses,⁸⁹⁻⁹¹ but not, as yet, to evaluate the effects of exercise.

Strain gauge transducers have also been secured on to the serosa of the GI segment to be studied; they measure mechanical instead of myoelectrical activity. They detect unidirectional, isometric muscle contractions and monitor the stress/tension applied by these muscle contractions on them. Colonic MMCs have been evaluated during long-distance exercise in dogs using this method.⁹² As with the serosally applied electrodes, this technique has been used successfully in horses,^{93,94} but never in association with exercise.

Manometers and transducers are used to measure changes in intraluminal pressure, which are interpreted as contractions that generate them. This technique has been used to study changes in the small intestinal MMC in dogs during exercise.³⁹ Unlike the methods mentioned above, this technique does not need surgical implantation so long as the GIT section of interest is accessible by either mouth or anus.

Mucosal permeability and absorption

Intestinal permeability can be assessed non-invasively in vivo by *orally administered markers*. These markers are cleared unaltered by renal excretion, wherein their concentrations in urine can be quantified over a specified post-feeding time period to determine the amount absorbed. Appearance, or not, of these markers in the urine will depend on the integrity of the intestinal mucosa,⁹⁵ since they are not absorbed through the intact mucosa.

Polyethylene glycol (PEG) polymers of different molecular weights have been widely used for assessment of permeability. This marker is also used to determine net water absorption, which is calculated directly by changes in PEG concentration within the

gut contents.⁹⁶ PEGs of high molecular weights have also been used as markers of gastrointestinal transit time in the horse.⁹⁷ PEG is not, however, an ideal marker for these kinds of study, since its purported size can be somewhat variable, and too large a dose can induce an osmotic hypersecretion.⁹⁸

Sugars are also commonly used as probes to evaluate intestinal integrity. Disaccharides, such as lactulose, and monosaccharides, such as L-rhamnose, D-xylose, 3-O-L-methyl M-glucose, and mannitol, are used to assess both transcellular and paracellular transport capacity across the intestinal mucosa. Their natural transport across the epithelium may be impaired by cellular damage, whereas necrosis may facilitate their passive movement into the bloodstream due to compromise of the mucosal barrier. Sugars have been used in humans to study the effect of running and cycling on intestinal transport.^{99,100} Agents such as these are preferable over PEG because of their uniformity of respective size, making them better discriminators at specific sites of GIT permeability. However, results of these tests should be regarded with caution when they are applied to the horse, since sugar absorption may be highly variable among individuals.⁸⁴ This variability most probably originates from regional variations in the GIT microbial population among horses, particularly in the stomach.⁸³

Finally, isotopic tracers like ⁵¹Cr-labeled ethylenediaminetetra-acetic acid (⁵¹Cr-EDTA) and ^{99m}Tc-diethylenetriaminopenta-acetate (^{99m}Tc-DTPA) can be added to drinks and used to measure unidirectional flux, from the intestinal lumen to the vascular compartment. Although the activity of these tracers in blood is readily measured, their radioactive character is a clear disadvantage,⁹⁵ largely limiting application to a laboratory setting.

Exercise and gastrointestinal function: what is known in other species that is pertinent to horses

Gastric motility and emptying

Different GI symptoms occur in as many as 30–50% of human participants in endurance events.^{101,19,102} Lower GI symptoms, which include diarrhea, rectal incontinence, urge to defecate, rectal bleeding, and abdominal cramps, are more common than upper GI

symptoms, such as gastroesophageal reflux, nausea, vomiting, and stomach pain. A direct effect of exercise on GI motility has been hypothesized to explain many of these disorders. Because of this high prevalence and the severity of some of the symptoms, many experiments have focused on the study of gastrointestinal motor function and transit. In the horse, exercise does not seem to result directly in signs of GI disturbance, but this observation should not exclude the possibility of GI alterations in response to the high demand imposed on the horse's body. Dehydration, musculoskeletal, or metabolic disorders may mask mild GI tract dysfunction during long, intense exercise.

During moderate exercise (less than 75% $\dot{V}O_{2\max}$), gastric emptying in man occurs at a rate similar to or slightly greater than that during rest.¹⁰³ However, more intense exercise appears to inhibit gastric emptying.^{18,103} In equine events where oral supplementation with electrolyte and carbohydrate solutions may be important to compensate a deficit of water, electrolytes, and carbohydrates, the rate of gastric emptying may limit the availability of administered fluids.¹⁰⁴ Solutions with higher carbohydrate concentration seem to delay gastric emptying in humans,³⁷ although increasingly larger volumes leave the stomach at faster rates.^{36,105} Pyloric closure and reduced antral contractions, observed by ultrasound after exercise, may be in part responsible for the delay in gastric emptying in humans.⁷⁷ In horses, solid meals ingested before exercise may be retained within the stomach for prolonged periods, favoring their bacterial fermentation, with production of volatile fatty acids and lactic acid, which may be deleterious to the squamous gastric mucosa.¹⁰⁶ In contrast, gastric emptying (GE) of fluid does not seem to be affected by exercise. Sosa et al³⁸ reported that GE of a single dose of isotonic fluid administered after intense exercise (70% $\dot{V}O_{2\max}$) in the horse did not differ from rest, which suggests that GE is not a limitation for rehydration. However, as discussed by the authors of this study, the results were highly variable, suggesting high measurement error due to the technique.³⁸

Intestinal motility and transit

In humans, inactivity has often been considered a cause of constipation; conversely, physical activity seems to promote colonic transit. Soffer et al found that bicycle exercise could convert the motor-fed pattern of the small intestine into a migrating motor complex (MMC), and that this effect was intensity-

related, although there was no effect on oro-cecal transit time.⁸² In contrast, Moses et al^{107,108} observed that moderate and intense treadmill exercise delayed small intestinal transit in humans, and van Nieuwenhoven et al²⁰ documented increased oro-cecal transit time. Yet Dainese et al¹⁰⁹ found that mild physical activity enhanced intestinal gas propulsion. In a study performed in fed dogs, moderate treadmill exercise decreased jejunal myoelectric activity only when exercise was prolonged beyond 30 min, and this inhibitory effect continued during recovery.⁸⁸ Furthermore, prolonged exercise induced MMCs characteristic of the interdigestive motor pattern in the jejunum of fed dogs,⁸⁸ whereas it can interrupt MMCs in the stomach and duodenum of fasted dogs.³⁹ Therefore, exercise can alter motor activity, which may explain some of the GI symptoms associated with exercise in humans.

Increased stool frequency and diarrhea induced by strenuous exercise in humans have been attributed to accelerated colonic transit time, or alternatively, to changes in absorption/secretion by the intestinal epithelium.¹⁰¹ Acute graded bicycle exercise decreases colonic phasic motor activity in an intensity-dependent way. This may facilitate transit by offering less resistance to colonic flow. After the end of exercise, propagated activity is increased, and this also may enhance colonic propulsion.¹¹⁰ In fasted dogs, exercise

decreases the frequency of colonic MMCs, and stimulates non-migrating colonic contractile activity within 3 h after ingestion of a meal. Exercise also stimulates defecation, giant migrating contractions, and mass movements in the colon, in both fasted and post-prandial states.⁹²

Current knowledge regarding exercise-induced changes in GI motility in the horse is meager. Pearson & Merritt studied the effect of a 14 km walk in donkeys, and neither GI transit nor the digestibility of hay was affected.³³ On the other hand, Pagan et al showed that 8 km of trotting and galloping by a group of Thoroughbreds resulted in a small, but significant decrease in dry matter digestibility, whereas transit rates of both a forage diet and a forage/grain diet were increased at 24 h after the exercise.³⁵ Finally, Orton et al studied the effect of 12 km daily trotting in yearling horses. Exercise increased apparent digestibility of dry matter, and increased the transit rate of particulates, but decreased the transit rate of the fluid phase (Fig. 8.1.4).³⁴

As mentioned, release of catecholamines and β -endorphins into the bloodstream, and stimulation of the sympathetic nervous system occur in the horse and in other species in response to exercise. This effect correlates with intensity of exercise and diminishes with continued training.^{50–53} Each of the components of this stress response affects gastrointestinal motor

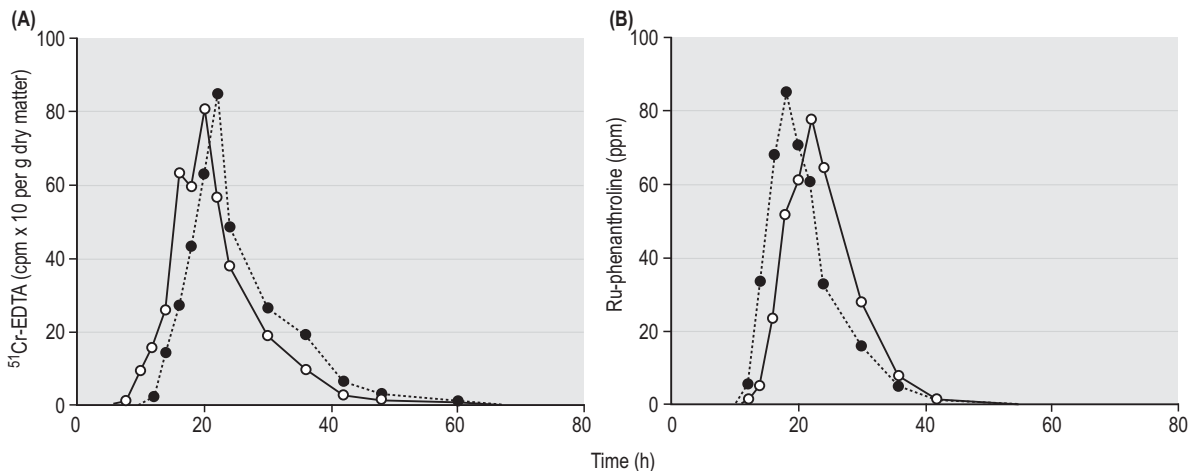


Fig. 8.1.4

Effect of exercise on rate of passage of fluid (A) and particulate (B) digesta. (A) Concentration of $^{51}\text{Cr-EDTA}$ in the feces of exercised (broken line) and non-exercised (solid line) yearling horses. Four animals per group. (B) Concentration of ruthenium (Ru)-phenanthroline in the feces of exercised (broken line) and non-exercised (solid line) yearling horses. Four animals per group. Exercise: 12 km trotting at 12 km/h. Transit of the fluid phase marker (A) was delayed in exercised horses, whereas transit of the particulate phase marker (B) was accelerated. These results are supported by Pagan et al,³² who observed that fecal excretion of the particulate marker ytterbium was accelerated in Thoroughbreds trotted and galloped for 8 km/day. (Reproduced with kind permission from Orton et al.³⁴)

function. Inhibition of gastric emptying and stimulation of colonic motor function are the characteristic pattern in response to different stressors. Central corticotropin-releasing hormone (CRH) seems to be a main component in the induction of this stress-related motor response. In some studies, CRH modulation of autonomic nervous system activity was found to be independent of activation of pituitary–adrenal hormone release.¹¹¹ Alexander et al measured plasma concentrations of CRH and adrenocorticotropin (ACTH) in the horse during and after acute intense exercise; ACTH was elevated, whereas CRF concentrations were not different from resting values.⁸ However, as suggested above, central rather than peripherally circulating CRH may be responsible for the gastrointestinal motor changes¹¹¹ and could be involved in the exercise-induced changes in GI transit documented in horses.^{34,35}

Gastrointestinal blood supply

Reduced splanchnic blood flow during high-intensity exercise may also play a role in slowing the rate of gastric emptying. Manohar et al reported a significant reduction in splanchnic blood flow during moderate exercise in the horse (Fig. 8.1.5).⁴⁶ Likewise, acute exercise decreased the clearance of BSP from the blood in an intensity-dependent manner in horses as a result of decreased splanchnic blood flow (Fig. 8.1.6).⁴⁹

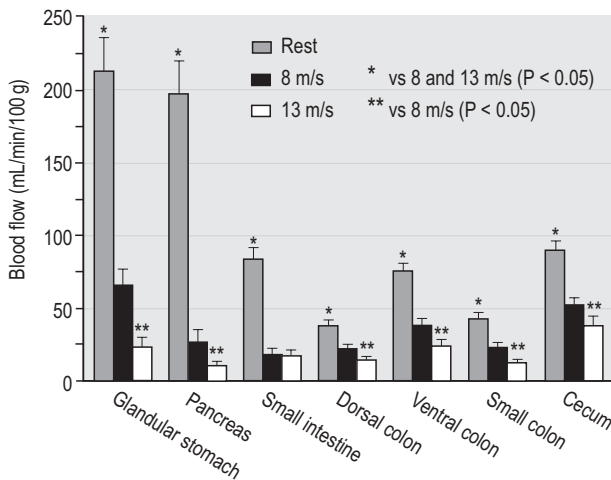


Fig. 8.1.5

Blood flow to pancreas and various gastrointestinal tract tissues in standing (rest) and exercised horses ($n = 9$ horses). (Reproduced with kind permission from Manohar et al.⁴⁶)

Splanchnic vasoconstriction in the horse may be induced by epinephrine (adrenaline) and norepinephrine (noradrenaline),⁷ or by release of hormones such as arginine vasopressin⁸ and VIP.⁵⁹ Endothelin, another potent vasoconstrictor released by endothelial cells, increases with exercise in humans¹¹² and horses.¹¹³ Its effect should be further investigated in horses, especially in view of a recent study that demonstrated a significant association between plasma endothelin-like immunoreactivity and pathogenesis of certain GIT disorders.¹¹⁴

Reduced blood flow during exercise also disrupts intestinal absorption. Carrier-mediated glucose uptake decreased with intense cycling in humans.¹¹⁵ This decrease in absorptive capacity may be a consequence of impaired blood flow and insufficient energetic supply for glucose active transport, which relies on ATPase activity, and could:

- prevent adequate plasma volume restitution, or
- increase osmotic pressure within the gut, which could result in diarrhea or loose stools.^{45,116}

On the other hand, 1 hour of exercise on a treadmill at 71% $\dot{V}O_{2\max}$ did not affect glucose, D-xylose, water, sodium, chloride, and bicarbonate intestinal uptake in

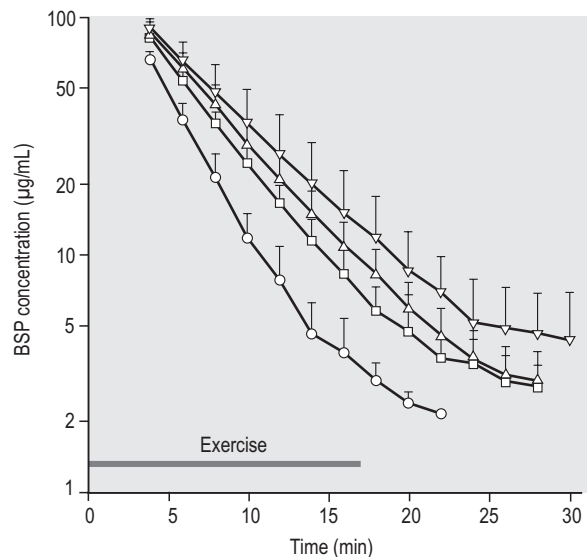


Fig. 8.1.6

Mean ($\pm$ SD) plasma concentrations of bromsulfalein (BSP) for eight horses exercising on a treadmill at four intensities. Bromsulfalein (5 mg/kg of bodyweight) was administered intravenously during a 45- to 60-second period. ○, resting; □, 40% $\dot{V}O_{2\max}$; △, 60% $\dot{V}O_{2\max}$; ▽, 80% $\dot{V}O_{2\max}$. (Reproduced with kind permission from Dyke et al.⁴⁹)

humans.¹¹⁷ In the horse, impaired glucose absorption could lead to its accumulation within the intestinal lumen, creating an osmotic load and/or an increase in the substrate available for bacterial digestion within the large intestine but it is unlikely that this would have any significant detrimental effects on the animal.

Mechanical factors

Mechanical bouncing of the body during exercise or compression of the viscera by abdominal muscles may also affect GI motility, since GI symptoms in humans occur more frequently during running than cycling events. Some cases of post-running volvulus have been reported in humans.¹¹⁶ Although light exercise has often been used as a way to facilitate passage of gas and promote transit in the large intestine of sick horses, strenuous exercise may promote excessive visceral movement. This, especially if combined with GIT dysmotility, could potentially promote intestinal displacement. The amount of colonic filling could have an influence on this bouncing effect.

Increased concentrations of prostaglandins and VIP, released by rubbing of the intestinal mucosa and distension,¹¹⁶ may shift intestinal absorption into secretion. Upregulated expression of these agents in the horse, especially within the distal large intestine, could theoretically result in diarrhea.

Mucosal barrier integrity

Fecal occult blood has been documented repeatedly in endurance athletes, but its etiology is still unknown. Yet the stomach is the most frequent site of running-associated hemorrhage in humans, possibly due to ischemic damage or trauma from the diaphragm.¹⁰¹ Bleeding may reflect pronounced tissue damage. Inadequate perfusion to the intestinal epithelium can compromise the protective barrier function. Compromised barrier function might also facilitate bacterial and endotoxin translocation and initiate a local immune response. Subsequent production and release of inflammatory mediators would exacerbate this whole process.^{118,119} Reduced mesenteric blood flow may also generate O₂ free radicals¹²⁰ which can also contribute to tissue damage.¹¹⁸ For example, running by some human subjects at 80% $\dot{V}O_{2\max}$ increased small intestinal permeability of lactulose compared with rest and running at 40 and 60% $\dot{V}O_{2\max}$. Since lactulose crosses

the epithelium only paracellularly, its increased transport is a strong indication of breakdown of the small intestinal barrier.¹²¹ To date, no studies have been done to investigate the effect of exercise on the integrity of the mucosal barrier in horses.

Clinical implications of exercise-associated changes in gastrointestinal function

As has already been alluded to, the anatomically and physiologically complex ceco-colon of horses could, in particular, predispose them to GIT disturbances during athletic training and competition. Undoubtedly a major confounding factor in this consideration is the feeding program, especially abrupt changes in diet composition or feeding routine. In fact, with respect to incidence of colic, the majority of the recent epidemiological studies more strongly indicate change in feeding program than type or duration of athletic activity as a high risk factor.^{122–126} Concerning the effect of exercise, Cohen et al¹²³ reported that primary use of the horse was not significantly associated with colic, although they then remarked that horses with colic were significantly more likely than control horses to have had a change in activity level during the 2-week period prior to examination. Likewise, Tinker et al¹²⁵ found that horses used for eventing or training had a significantly higher incidence rate of colic when compared to mature horses that were sedentary. This is not to say that the origin of the problem in all these cases was large intestinal (there was no information on site in the report) but, because of that organ's complexity, it must be considered as a highly likely origin nevertheless.

Anatomical considerations

From an anatomical standpoint, the fact that a large section of mid-colon floats freely within the abdominal cavity makes it prone to various forms of displacement that can result in serious colic. Whether any of these conditions may result from, for example, a combination of physical characteristics of the contents therein (e.g. collected sand; the gas:ingesta ratio) and type of exercise the horse is asked to perform remains to be established.

Physiological considerations

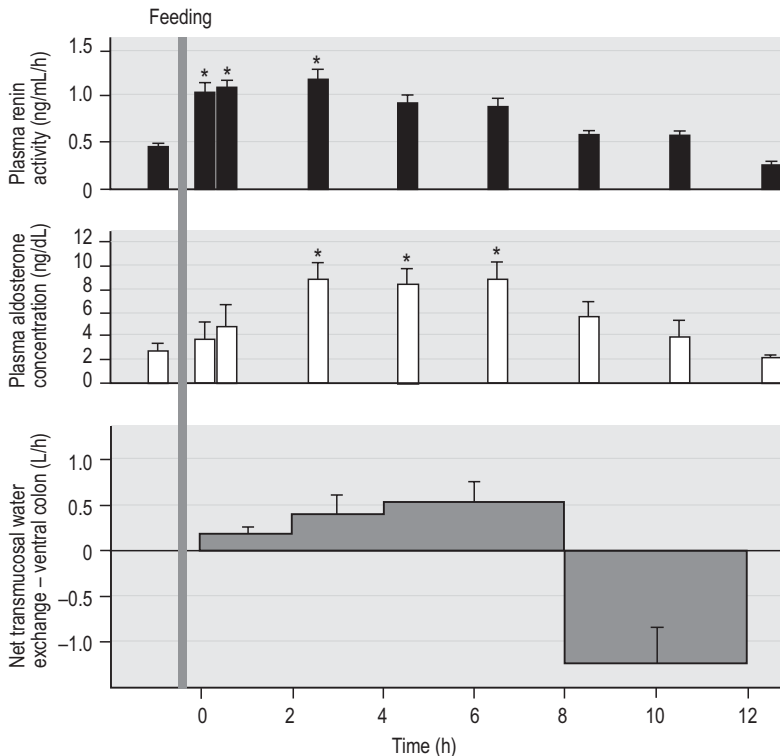
From a physiological standpoint, at least three areas deserve attention in future research on the effects of exercise on GIT function in horses.

The first concerns factors that determine daily net water movement across the gut mucosa, between lumen and plasma. It is a common practice to withhold food hours before an event to decrease gut fill, and consequently, bodyweight. However, eating before or during a competition may have beneficial effects, as observed in human endurance athletes.¹²⁷ Ingestion of food may attenuate the reduction in blood flow induced by moderate to severe exercise.¹²⁸ Accordingly, blood flow to the digestive tract during exercise seems to be higher in fed than fasted ponies.¹²⁸ Fed horses may start with elevated blood flow of the GI tract, so that the impact of exercise on blood distribution is not so marked. Ingestion of food is accompanied by saliva production, and gastric and pancreatic secretion. Forage stimulates more water ingestion and digestive secretions than concentrate meals.¹²⁹ Factors which determine daily net water flux across ceco-colonic mucosa are complex and are characterized by regional compartmentalization.¹³⁰ Such factors include timing and composition of ingested food which impact directly on fermentative activity, water and electrolyte status of the horse, and efficacy of GI motility. For instance, Clarke et al have shown that during early post-prandial stages of a large meal of balanced ration pellets, the VFA production from its fermentation within the colons may outstrip the subsequent VFA absorption, resulting in a large flux of water into the colonic lumen from the plasma space.¹³¹ This can deplete plasma volume to a point where renin-angiotensin and aldosterone are released to promote plasma water conservation, the degree of which may be great enough to result in significant rebound dehydration of distal colonic contents (Fig. 8.1.7).¹³² Combine a strenuous training session on top of this, and you have the makings of a serious impaction. In contrast, when that same meal was split into small volumes and given over 12 hours, mass movement of plasma water into the colons did not occur.¹³¹

Second, the effect of exercise on increasing GI mucosal permeability in humans has been discussed earlier in the chapter. Brouns & Becker¹²⁷ mention cramps, diarrhea (sometimes bloody), and urge to defecate as symptoms associated with exhausting endurance events. And, in the study by Ashton et al¹¹⁸ a majority of subjects reported 'chills and nausea' some time within the 24 h following the strenuous exercise

they had undergone. Both these studies suggest that exercise-related endotoxemia could account for the symptoms reported. It is safe to assume that there is, at any given time, a much greater load of endotoxin within the equine ceco-colonic lumen than in that of humans.¹³³ Detrimental effects of endotoxemia in horse are well recognized and include leucopenia, hypotension, and intestinal dysmotility and hypersecretion.^{133,134} Clearly, if athletic activities consistently caused signs indicative of endotoxemia in a large number of horses, the beast would not be used for the many things humans ask of it. However, it would still be important to know if exercise, especially if strenuous, does cause a degree of endotoxemia that could be clinically important.¹³⁵ This suggestion is further supported by the observation that physical stress enhances colonic epithelial permeability in rats via peripheral release of corticotrophin-releasing hormone (CRH).¹³⁶ Accordingly, Baker et al¹³⁷ reported in 1988 that a group of racing-fit horses had significantly higher serum anti-LPS concentrations than a similar group of horses that were not trained, suggesting that increased intestinal permeability could have occurred as a result of their repeated exertion. More studies following up on this important observation need to be done.

Third, the transient defecation of non-formed, sometimes quite watery feces from some horses when they are subjected to a training session or race environment is a well-recognized phenomenon. It is usually of little consequence to the horse, but can be very bothersome to owners and trainers, especially when it occurs during a public display. Commonly, it is attributed to 'nerves,' which could be one reasonable explanation. Nevertheless, this is an exercise-related event and it would be interesting to know its functional basis because it might be controllable. Probably the first place to look would be the small colon, where final desiccation of colonic contents takes place resulting in the formation of the fecal balls. This process involves a coordinated interaction between mechanisms that control motility and net transmucosal water flux; this is undoubtedly closely monitored by the enteric nervous system, much of which we still need to learn about with respect to equine small colon function. What we do know is that at least some of the water absorption is determined by a Na^+/K^+ -ATPase pump, the functional status of which is under the control of aldosterone.²⁸ It seems unlikely, however, that the transient, exercise-related loose feces problem would be due to a downregulation of this system. More likely possibilities would be either a

**Fig. 8.1.7**

Post-prandial activation of the renin–angiotensin–aldosterone system superimposed on net fluid transport in pony ventral colon. Rapid accumulation of bacterially produced volatile fatty acids within the colon after ingestion of a single large meal results in net flux of water from the plasma compartment into the colonic lumen. In response to this plasma volume depletion, renin–angiotensin and aldosterone are released to promote water absorption from the luminal compartment back into the vascular system. Addition of strenuous exercise during this period could exacerbate dehydration of colonic contents, and therefore, increase the risk of colonic impaction. (*Significantly ($P < 0.05$) greater than preceding value.) (Reproduced with kind permission from White.¹³⁰)

small colon dysmotility that allows more rapid passage of contents through the organ, or the upregulation of

a secretagogue such as CRH, VIP, or some combination thereof.^{59,111,135,136}

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